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Certified By

Jon W Dudas

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020304

17607 U.S. PTO

PTO/SB/16 (08-03)

Approved for use through 07/31/2006. OMB 0651-0032

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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020304

INVENTOR(S)					
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Guido		Grandi		Milano, Italy	
Additional inventors are being named on the <u>second</u> separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Immunogenic Compositions For Streptococcus Pyogenes					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number: <u>27476</u>					
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ENCLOSED APPLICATION PARTS (check all that apply)					
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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.					
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input type="checkbox"/> No.					
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[Page 1 of 2]

Respectfully submitted,

SIGNATURE

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Date

Feb 3, 2004REGISTRATION NO. 45,680

(if appropriate)

Docket Number: 20663.002**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

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Additional Page

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Docket Number 20663.002

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Number 2 of 2

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IMMUNOGENIC COMPOSITIONS FOR *STREPTOCOCCUS PYOGENES*

This application incorporates by reference in its entirety U.S. provisional patent application No. 60/491,822, filed on July 31, 2003.

TECHNICAL FIELD

5 This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens derived from *Streptococcus pyogenes* and their use in immunisation. All documents cited herein are incorporated by reference in their entirety.

BACKGROUND ART

10 Group A streptococcus ("GAS", *S.pyogenes*) is a frequent human pathogen, estimated to be present in between 5-15% of normal individuals without signs of disease. When host defences are compromised, or when the organism is able to exert its virulence, or when it is introduced to vulnerable tissues or hosts, however, an acute infection occurs. Related diseases include puerperal fever, scarlet fever, erysipelas, pharyngitis, impetigo, necrotising fasciitis, myositis and streptococcal toxic shock syndrome.

15 Although *S.pyogenes* may be treated using antibiotics, a prophylactic vaccine to prevent the onset of disease is desired. Efforts to develop such a vaccine have been ongoing for many decades. While various GAS vaccine approaches have been suggested and some approaches are currently in clinical trials, to date, there are no GAS vaccines available to the public.

20 It is an object of the invention to provide further and improved compositions for providing immunity against GAS disease and/or infection. The compositions are based on a combination of two or more (e.g. three or more) GAS antigens.

DISCLOSURE OF THE INVENTION

25 Applicants have discovered a group of thirty GAS antigens that are particularly suitable for immunisation purposes, particularly when used in combinations. In addition, Applicants have identified a GAS antigen (GAS 40) which is particularly immunogenic used either alone or in combinations with additional GAS antigens.

30 The invention therefore provides an immunogenic composition comprising GAS 40, a fragment thereof or a polypeptide having sequence identity thereto. The invention further includes an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to ten GAS antigens, wherein said combination includes GAS 40 or a fragment thereof or a polypeptide having sequence identity thereto. Preferably, the combination consists of three, four, five, six, or seven GAS antigens. Still more preferably, the combination consists of three, four, or five GAS antigens.

35 The invention also provides an immunogenic composition comprising a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of a first antigen group, said

first antigen group consisting of: GAS 117, GAS 130, GAS 277, GAS 236, GAS 40, GAS 389, GAS 504, GAS 509, GAS 366, GAS 159, GAS 217, GAS 309, GAS 372, GAS 039, GAS 042, GAS 058, GAS 290, GAS 511, GAS 533, GAS 527, GAS 294, GAS 253, GAS 529, GAS 045, GAS 095, GAS 193, GAS 137, GAS 084, GAS 384, GAS 202, and GAS 057. These antigens are referred to herein as the 'first antigen group'. Preferably, the combination of GAS antigens consists of three, four, five, six, seven, eight, nine, or ten GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens consists of three, four, or five GAS antigens selected from the first antigen group.

GAS 39, GAS 40, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511 are particularly preferred GAS antigens. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination includes GAS 40.

Representative examples of some of these antigen combinations are discussed below.

The combination of GAS antigens may consist of three GAS antigens selected from the first antigen group. Accordingly, in one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and a third GAS antigen selected from the first antigen group. Preferred combinations include GAS 40, GAS 117 and a third GAS antigen selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and two additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and two GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In another embodiment, the combination of GAS antigens consists of GAS 117 and two additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of four GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and two additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40, GAS 117, and two GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and three additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and three additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In one embodiment, the combination of GAS antigens consists of GAS 117 and three additional antigens selected from the first antigen group.

The combination of GAS antigens may consist of five GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and three additional GAS antigens selected from the first antigen group. Preferred combinations

include GAS 40, GAS 117 and three additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511.

In another embodiment, the combination of GAS antigens consists of GAS 40 and four additional GAS antigens selected from the first antigen group. Preferred combinations include GAS 40 and four additional GAS antigens selected from the group consisting of GAS 39, GAS 57, GAS 117, GAS 202, GAS 294, GAS 527, GAS 533, and GAS 511. In one embodiment, the combination of GAS antigens consists of GAS 117 and four additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of eight GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and six additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40 and seven additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 117 and seven additional GAS antigens selected from the first antigen group.

The combination of GAS antigens may consist of ten GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40, GAS 117 and eight additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 40 and nine additional GAS antigens selected from the first antigen group. In one embodiment, the combination of GAS antigens consists of GAS 117 and nine additional GAS antigens selected from the first antigen group.

Each of the GAS antigens of the first antigen group are described in more detail below. Genomic sequences of at least three GAS strains are publicly available. The genomic sequence of an M1 GAS strain is reported at Ref. 1. The genomic sequence of an M3 GAS strain is reported at Ref. 2. The genomic sequence of an M18 GAS strain is reported at Ref. 3. Preferably, the GAS antigens of the invention comprise polynucleotide or amino acid sequence of an M1, M3 or M18 GAS strains. More preferably, the GAS antigens of the invention comprise a polynucleotide or amino acid sequence of an M1 strain.

(1) GAS 117

GAS 117 corresponds to M1 GenBank accession numbers GI:13621679 and GI:15674571, to M3 GenBank accession number GI:21909852, to M18 GenBank accession number GI: 19745578, and is also referred to as 'Spy0448' (M1), 'SpyM3_0316' (M3), and 'SpyM18_0491' (M18). Examples of amino acid and polynucleotide sequences of GAS 117 of an M1 strain are set forth below:

SEQ ID NO: 1

MTLKKHYLLSLLALVTVGAAFNSTQSVSAQVYSNEGYHQHLTDEKSHLQYSKDNAQLQLRNILDGYQND
LGRHYSSYYYNLRTVMGLSSEQDIEKHYEELKNKLHDMYNHY

SEQ ID NO: 2

ATGACACTAAAAAACACTATTATCTTCTCAGCCTGCTAGCTCTTGTAACGGTTGGTGCTGCCTTTAACA

CAAGCCAGAGTGTCAAGTGCACAAGTTTATAGCAATGAAGGGTATCACCAGCATTGACTGATGAAAAATC
ACACCTGCAATATAGTAAAGACAACGCACAACCTTCAATTGAGAAATATCCTTGACGGCTACCAAAATGAC
CTAGGGAGACACTACTCTAGCTATTATTACTACAACCTAAGAACCGTTATGGGACTATCAAGTGAGCAAG
ACATTGAAAAACACTATGAAGAGCTTAAGAACAAGTTACATGATATGTACAATCATTATTAA

Preferred GAS 117 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 1; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 1, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 117 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 1. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 1. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 1. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 1 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide; of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(2) GAS 130

GAS 130 corresponds to M1 GenBank accession numbers GI:13621794 and GI:15674677, to M3 GenBank accession number GI: 21909954, to M18 GenBank accession number GI: 19745704, and is also referred to as 'Spy0591' (M1), 'SpyM3_0418' (M3), and 'SpyM18_0660' (M18). GAS 130 has potentially been identified as a putative protease. Examples of amino acid and polynucleotide sequences of GAS 130 of an M1 strain are set forth below:

SEQ ID NO: 3

MSHMKKRPEVLSPAGTLEKLKVAIDYGADAVFVGGQAYGLRSRAGNFSMEELQEGIDYAHARGAKVYVAA
NMVTHEGNÉIGAGEWFRQLRDMGLDAVIVSDPALIVICSTEAPGLEIHLSTQASSTNYETFEFWKAMGLT
RVVLAREVNMAELAEIRKRTDVEIEAFVHGAMCISYSGRCVLSNHMSHRDANRGGCSQSCRWKYDLYDMP
FGGERLSLKGEIPEDYSMSSVDMCMIDHIPDLIENGVDLSKIEGRMKSIHYVSTVTNICYKAAVGAYMES P
EAFYAIKEELIDELWKVAQRELATGFYGIPTENEQLFGARRKI PQYKFVGEVVAFDSASMTATIRQRNV
IMEGDRIECYGPGRHFETVVKDLHDADGQKIDRAPNPMELLTISLPREVKPGDMIRACKEGLVNLYQKD
GTSKTVRT

SEQ ID NO: 4

ATGTCACATATGAAAAACGTCCCGAGGTCTTATCACCTGCTGGAACACTTGAAAAATTAAAGTTGCGA
TTGACTATGGCGCAGATGCTGTTTTTGTGAGGGCAGGCCTATGGCCTAAGAAGCCGCGCTGGTAACTT
CTCTATGGAAGAATTGCAAGAAGGCATTGATTATGCACATGCGCGTGGAGCTAAGGTCTATGTTGCTGCT
AACATGGTTACCCACGAAGGGAACGAAATTGGTGCGGGCAGTGTTTCGTCAACTGCGTGATATGGGGC
TTGATGCGGTCATTGTTTCAGATCCAGCCTTGATTGTTATTTGTTCAACAGAAGCCCCAGGTTTGGAAT
TCATTTGTCAACGCAAGCTTCATCTACCAATTACGAGACCTTTGAATTTTGGAAGCCATGGGCTTGACC
CGAGTTGTTTTAGCTCGCGAGGTTAATATGGCCGAGTTAGCAGAAATCCGCAAGCGGACAGATGTGGAAA
TTGAAGCCTTTGTCCATGGAGCCATGTGTATCTCTATTACAGGCCGCTGTGTTTTGTCAAACCATGAG
TCACCGTGATGCCAACAGGGGCGGCTGCTCACAGTCTTGCCGCTGGAAGTATGATTTGTATGACATGCCA
TTTGAGGAGAGCGCCGCTCCTTAAAGGGGAAATTCAGAAGACTATTCTATGTCTCTGTGACATGT
GTATGATTGACCATATTCCTGACCTGATTGAAATGGGGTTGATAGCTTAAAAATTGAAGGCCGAATGAA
ATCTATCCACTACGTCTCAACCGTAACCACTGTTACAAGGCGGCTGTAGGTGCTTACATGGAAAGCCCA
GAAGCTTTTTATGCTATCAAAGAGGAATTGATTGACGAGTTGTGGAAGGTTGCCCGAGCGAGTTGGCTA
CAGGTTTTTACTATGGTATCCCAACTGAAATGAACAATTATTTGGTGCTCGCCGCAAAATTCACAATA
TAAATTTGTGCGGAGAAGTAGTTGCCTTTGACTCAGCTAGCATGACAGCGACCATTCGTCAGCGTAATGTC
ATCATGGAAGGCGATCGGATTGAATGTTATGGACCAGGTTTCCGTCATTTTGAAACGGTTGTTAAGGACT

TACATGATGCGGATGGCCAAAAGATTGACCGTGCCCCAAATCCAATGGAACCTCTTAACCATCTCTTTACC
GAGAGAAGTTAAGCCAGGGGATATGATTAGGGCTTGCAAGGAAGGTCTGGTTAACCTCTATCAAAAAGAT
GGCACCAGTAAACTGTTAGAACATAG

- 5 Preferred GAS 130 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 3; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 3, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 130 proteins include variants (e.g. allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 3. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 3. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 3. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(3) GAS 277

GAS 277 corresponds to M1 GenBank accession numbers GI:13622962 and GI:15675742, to M3 GenBank accession number GI: 21911206, to M18 GenBank accession number GI: 19746852, and is also referred to as 'Spy1939' (M1), 'SpyM3_1670' (M3), and 'SpyM18_2006' (M18). Amino acid and polynucleotide sequences of GAS 277 of an M1 strain are set forth below:

SEQ ID NO: 5

MTTMQKTI SLLSLALLIGLLGTSGKAISVYAQDQHTDNVIAESTISQVSVEASMRGTEPYIDATVTTDQP
VRQPTQATITLKDASDNTINSWVYTMAAQRRFTAWFDLTGQKSGDYHVTVTVHTQEKA VTGQSGTVHFD
QNKARKTPTNMQQKDTSKAMTNSVDVDTKAQTNQSANQEBIDSTSNPFRSATNHRSTSLKRSTKNEKLTP
ASNSQKNGSNKTKMLVDKEEVKPTSKRGFPWVLLGLVSLAAGLFIAIQKVSRRK

SEQ ID NO: 6

ATGACAACTATGCAAAAAACAATTAGCTTATTATCACTAGCTTTACTTATTGGTTTGCTGGGGACTTCTG
GCAAAGCCATATCTGTGTATGCACAAGATCAGCACACTGATAATGTTATAGCTGAATCAACTATTAGTCA
GGTCAGTGTTGAAGCCAGTATGCGTGGAACAGAACCTTATATTGATGCTACAGTCACCACAGATCAACCT
GTCAGACAACCAACTCAGGCAACGATAACACTTAAAGACGCTAGTGATAATACTATTAATAGTTGGGTAT
ATACTATGGCAGCGCAACAGCGTCGTTTACAGCTTGGTTTGATTAACTGGACAAAAGAGTGGTGACTA
TCATGTAACCTGTCACCGTTCATACTCAAGAAAAGGCAGTAACCTGGTCAATCAGGAAGTTCATTTTGAT
CAAAACAAAGCTAGAAAAACACCAACTAATATGCAACAAAAGGATACTTCTAAAGCAATGACGAATTCAG
TCGATGTAGACACAAAAGCTCAAAACAAATCAATCAGCTAACCAAGAAATAGATTCTACTTCAAATCCTTT
CAGATCAGCTACTAATCATCGATCAACTTCCTTAAAGCGATCTACTAAAAATGAGAACTTACACCAACT
GCTAGTAATAGCCAAAAAACGGTAGCAACAAGACAAAATGCTAGTGGACAAAGAGGAAGTAAACCTA
CTTCAAAAAGAGGATTCCCTTGGGTCTTATTAGGTCTAGTAGTCAGTTTAGCTGCAGGTTTATTTATAGC
TATTCAAAAAGTATCTAGACGAAAATAA

- Preferred GAS 277 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 5; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 5, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 277 proteins include variants (e.g. allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 5. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 5. Other preferred fragments lack one or more amino acids

(e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 5. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 5 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(4) GAS 236

GAS 236 corresponds to M1 GenBank accession numbers GI:13622264 and GI:15675106, M3 GenBank accession number GI: 21910321, and to M18 GenBank accession number GI: 19746075, and is also referred to as 'Spy1126' (M1), 'SpyM3_0785' (M3), and 'SpyM18_1087' (M18). Amino acid and polynucleotide sequences of GAS 236 from an M1 strain are set forth below:

SEQ ID NO: 7

MTQMNYTGKVKRVAIIANGKYQSKRVASKLFSVFKDDPDFYLSKKNPDIVISIGGDGMLLSAFHMYEKEL
DKVRFVGIHTGHLGFYTDYRDFEVDKLIDNLRKDKGEQISYPILKVAITLDDGRVVKARALNEATVKRIE
KTMVADVIIHVKFESFRGDGIVSVSTPTGSTAYNKSLGGAVLHPTIEALQLTEISSLNRRVFRITLGSSII
IPKKDKIELVPKRLGIYITISIDNKTYQLKNVTKVEYFIDDEKIHVSSPSHTSFWERVKDAFIGEIDS

SEQ ID NO: 8

ATGACACAGATGAATTATACAGGTAAGGTAAAACGAGTTGCTATTATTGCAAATGGTAAGTACCAAAGTA
AACGCGTCGCCTCCAACTTTTCTCCGTATTTAAAGATGATCCTGATTTCTATCTTTCAAAGAAAAATCC
GGATATTGTGATTTCTATTGGCGGAGATGGGATGCTCTTATCTGCCTTTCACATGTATGAAAAAGAATTA
GATAAGGTACGTTTGTAGGAATCCACACCGGTCTCTTGGCTTTTATACCGATTATAGGGATTTTGAAG
TTGATAAATTAATTGATAATTTAAGAAAAGACAAGGGAGAACAAATCTCTTATCCGATTTTAAAAGTTGC
TATTACTTTAGATGATGGTCGTGTGGTTAAAGCGCGTGCTTTGAATGAAGCGACGGTTAAGCGTATTGAA
AAAACGATGGTAGCAGATGTTATTATTAACCATGTCAAATTTGAAAGCTTCCGAGGTGATGGGATTTTCAG
TATCGACCCCGACAGGGAGCACAGCCTACAATAAATCTTTAGGTGGTGTCTTGCATCCGACGATTGA
AGCGCTGCAATTGACGGAAATTTCCAGTCTTAATAACCGTGTCTTTAGAACCTTGGGCTCATCAATCATT
ATTCCCAAAAAGATAAGATTGAGTTAGTGCCAAAACGATTAGGAATTTATACCATTTCCATTGATAATA
AAACCTATCAGTTAAAAAATGTGACGAAGGTGGAGTATTTTATCGACGATGAGAAAATTCATTTTGTTC
CTCTCCGAGTCATACGAGCTTTTGGGAAAGGGTCAAGGATGCCTTTATTGGAGAGATTGACTCATGA

Preferred GAS 236 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 7; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 7, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 236 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 7. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 7. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 7. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 7 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(5) GAS 040

GAS 040 corresponds to M1 GenBank accession numbers GI:13621545 and GI:15674449, to M3 GenBank accession number GI: 21909733, to M18 GenBank accession number GI:19745402, and is

also referred to as 'Spy0269' (M1), 'SpyM3_0197' (M3), 'SpyM18_0256' (M18) and 'prgA'. GAS 040 has also been identified as a putative surface exclusion protein. Amino acid and polynucleotide sequences of GAS 040 from an M1 strain are set forth below:

SEQ ID NO: 9

5 MDLEQTKPNQVKQKIALTSTIALLSASVGVSHQVKADDRASGETKASNTHDDSLPKPETIQEAKATIDAV
EKTLSQQKABTELATALTCTTAEINHLKEQQDNEQKALTSABEYITNTLASSEETLLAQGAHQRELT
TETELHNAQADQHSKETALSEQKASISAETTRAQDLVEQVKTSEQNI AKLNAMISNPDAITKAAQTANDN
TKALSSELEKAKADLENQKAKVKKQLTEELAAQKAALAEKEAELSRLKSSAPSTQDSIVGNNTMKAPQGY
10 PLEELKKLEASGYIGSASYNYYKEHADQIIAKASPGNQLNQYQDIPADRNRFDVDPDLTPEVQNELAQF
AAHMINSVRRQLGLPPVTVTAGSQBFARLLSTSYKKTHGNTRPSFVYGQPGVSGHYGVGPHDKTIIEDSA
GASGLIRNDDNMYENIGAFNDVHTVNGIKRGIYDSIKYMLFTDHLHGNTYGHAINFLRVDKHNPNAPVYL
GFSTSNVGSLSNEHFVMPESNIAHQRFNKTPIKAVGSTKDYAQRVGTVSDTIAAIKGVSSLENRLSAI
HQEADIMAAQAKVSQQLQGLASTLKQSDSLNLQVRQLNDTKGSLRTELLAAKAKQAQLEATRDQSLAKLA
15 SLKAALHQTEALAEQAAARVTALVAKKAHLQYLRDFKLNPNRLQVIRERIDNTKQDLAKTTSSLLNAQEA
LAALQAKQSSLEATIATTEHQLTLLKTLANEKEYRHLDEDIATVPDLQVAPPLTGKPLSYSKIDTTPLV
QEMVKETKQLEASARLAAENTSLVAEALVGQTSSEMVASNAIVSKITSSITQPSSKTSYSGSGSSTTSNLI
SDVDESTQRALKAGVVMLAAVGLTGFRFRKESK

SEQ ID NO: 10

20 ATGGACTTAGAACAAACGAAGCCAAACCAAGTTAAGCAGAAAATTGCTTTAACCTCAACAATTGCTTTAT
TGAGTGCCAGTGTAGGCGTATCTCACCAAGTCAAAGCAGATGATAGAGCCTCAGGAGAAACGAAGGCGAG
TAATACTCAGCAGATAGTTTACCAAACAGAAACAATTCAAGAGGCAAAGGCAACTATTGATGCAGTT
GAAAAAATCTCAGTCAACAAAAGCAGAACTGACAGAGCTTGCTACCGCTCTGACAAAACTACTGCTG
25 AAATCAACCACTTAAAGAGCAGCAAGATAATGAACAAAAGCTTTAACCTCTGCACAAGAAATTTACAC
TAATACTCTTGCAAGTAGTGAGGAGACGCTATTAGCCCAAGGAGCCGAACATCAAAGAGAGTTAACAGCT
ACTGAAACAGAGCTTCATAATGCTCAAGCAGATCAACATTCAAAGAGACTGCATTGTCAGAACAAAAG
CTAGCATTTTCAGCAGAACTACTCGAGCTCAAGATTTAGTGGAACAAGTCAAACGCTCTGAACAAAATAT
TGCTAAGCTCAATGCTATGATTAGCAATCCTGATGCTATCACTAAAGCAGCTCAAACGGCTAATGATAAT
ACAAAAGCATTAAAGCTCAGAAATTGGAGAAGGCTAAAGCTGACTTAGAAAATCAAAAAGCTAAAGTTAAA
30 AGCAATTGACTGAAGAGTTGGCAGCTCAGAAAGCTGCTCTAGCAGAAAAAGAGGCAGAACTTAGTCGTCT
TAAATCCTCAGCTCCGTCTACTCAAGATAGCATTGTGGGTAAATAACCATGAAAGCACCGCAAGGCTAT
CCTCTTGAAGAACTTAAAAAATTAGAAGCTAGTGGTTATATTGGATCAGCTAGTTACAATAATTATTACA
AAGAGCATGCAGATCAAAATTATTGCCAAAGCTAGTCCAGGTAATCAATTAAATCAATACCAAGATATTCC
AGCAGATCGTAATCGCTTTGTTGATCCCGATAATTTGACACCAGAAGTGCAAAATGAGCTAGCGCAGTTT
35 GCAGCTCACATGATTAAAGTGTAAAGACAATTAGGTCTACCACCAGTTACTGTTACAGCAGGATCAC
AAGAAATTTGCAAGATTACTTAGTACCAGCTATAAGAAAATCATGGTAATACAAGACCATCATTTGTCTA
CGGACAGCCAGGGGTATCAGGGCATTATGGTGTGGGCTCATGATAAACTATTATTGAAGACTCTGCC
GGAGCGTCAGGGCTCATTGCAATGATGATAACATGTACGAGAATATCGGTGCTTTTAACTGATGTGCATA
CTGTGAATGGTATTAAACGTGGTATTTATGACAGTATCAAGTATATGCTCTTTACAGATCATTTACACGG
40 AAATACATACGGCCATGCTATTAACTTTTTACGTGTAGATAAACATAACCCTAATGCGCCTGTTTACCTT
GGATTTTCAACCAGCAATGTAGGATCTTTGAATGAACACTTTGTAATGTTTCCAGAGTCTAACATTGCTA
ACCATCAACGCTTTAATAAGACCCCTATAAAAGCCGTTGGAAGTACAAAAGATTATGCCCAAAGAGTAGG
CACTGTATCTGATACTATTGCAGCGATCAAAGGAAAAGTAAGCTCATTAGAAAATCGTTTGTGCGCTATT
CATCAAGAAGCTGATATTATGGCAGCCCAAGCTAAAGTAAGTCAACTTCAAGGTAAATTAGCAAGCACAC
45 TTAAGCAGTCAGACAGCTTAAATCTCAAGTGAGACAATTAAATGATACTAAAGGTTCTTTGAGAACAGA
ATTACTAGCAGCTAAAGCAAAACAAGCACAACTCGAAGCTACTCGTGATCAATCATTAGCTAAGCTAGCA
TCGTTGAAAGCCGCACTGCACCAGACAGAAGCCTTAGCAGAGCAAGCCGAGCCAGAGTGACAGCACTGG
TGGCTAAAAAAGCTCATTGCAATATCTAAGGGACTTTAAATTGAATCCTAACCGCCTTCAAGTGATACG
TGAGCGCATTGATAATACTAAGCAAGATTGGCTAAAACTACCTCATCTTTGTTAAATGCACAAGAAGCT
50 TTAGCAGCCTTACAAGCTAAACAAAGCAGTCTAGAAGCTACTATTGCTACCACAGAACACCAAGTTGACTT
TGCTTAAACCTTAGCTAACGAAAAGGAATATCGCCACTTAGACGAAGATATAGCTACTGTGCTGATTT
GCAAGTAGCTCCACCTCTACGGGCGTAAACCGCTATCATATAGTAAGATAGATACTACTCCGCTTGTT
CAAGAAATGGTTAAAGAAACGAAACAACCTATTAGAAGCTTCAGCAAGATTAGCTGCTGAAAATACAAGTC
TTGTAGCAGAAGCGCTTGTTGGCCAAACCTCTGAAATGGTAGCAAGTAATGCCATTGTGTCTAAAATCAC
55 ATCTTCGATTACTCAGCCCTCATCTAAGACATCTTATGGCTCAGGATCTTCTACAACGAGCAATCTCATT
TCTGATGTTGATGAAAGTACTCAAAGAGCTCTTAAAGCAGGAGTCGTATGTTGGCAGCTGTGCGCCTCA
CAGGATTTAGGTTCCGTAAGGAATCTAAGTGA

Preferred GAS 040 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 9; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 9, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These GAS 040 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 9. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 9. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 9. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 9 is removed. As another example, in one embodiment, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 9 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

Further illustration of domains within GAS 40 is shown in FIGURES 1 and 2. As shown in these figures, GAS 40 contains a leader peptide sequence within amino acids 1 – 26, a coiled-coil region within amino acids 58 – 261, a coiled coil region within amino acids 556 – 733, a leucine zipper region within amino acids 673 – 701 and a transmembrane region within amino acids 855 – 866.

The coiled-coil regions of GAS 40 are likely to be involved in the formation of oligomers such as dimers or trimers. Such oligomers could be homomers (containing two or more GAS 40 proteins oligomerized together) or heteromers (containing one or more additional GAS proteins oligomerized with GAS 40).

Accordingly, in one embodiment, the combinations of the invention include a GAS 40 antigen in the form of an oligomer. The oligomer may comprise two more GAS 40 antigens or fragments thereof, or it may comprise GAS 40 or a fragment thereof oligomerized to a second GAS antigen. Preferably, a GAS 40 fragment used within an oligomer includes a portion of one of the coiled coil or leucine zipper domains.

(6) GAS 389

GAS 389 corresponds to M1 GenBank accession numbers GI:13622996 and GI:15675772, to M3 GenBank accession number GI: 21911237, to M18 GenBank accession number GI: 19746884, and is also referred to as 'Spy1981' (M1), 'SpyM3_1701' (M3), 'SpyM18_2045' (M18) and 'relA'. GAS 389 has also been identified as a (p)ppGpp synthetase. Amino acid and polynucleotide sequences of GAS 389 from an M1 strain are set forth below:

SEQ ID NO: 11

MRNEMAKIMNVTGEEVIALAATYMTKADVAFVAKALAYATAAHFYQVRKSGEPYIVHPIQVAGILADLHL
DAVTVACGFLHDVVEDTDITLDEIEADFGHDARDIVDGVTKLGEVEYKSHEEQLAENHRKMLMAMSKDIR
VILVKLADRLHNMRTLKHLRKDKQERISRETMEIYAPLAHRLGISRIKWELEDLAFRYLNETEFYKISHM
MKEKRREREALVEAIVSKVKTYTTQQGLFGDVYGRPKHIYSIYRKM RDKKRFDQIFDLIAIRCVMETQS
DVYAMVGYIHELWRPMPGRFKDYIAAPKANGYQSIHTTVYGPKGPIEQIRTKDMHQVABYGVAHWAYK

KGVRGKVNQAEQAVGMNWIKELEVELQDASNGDAVDFVDSVKEDIFSERIYVFTPTGAVQELPKESGPIDF
AYAIHTQIGEKATGAKVNGRMVPLTAKLKTGDVVEIITNANSFGPSRDWVKLVKTNKARNKIRQFFKNQD
KELSVNKGRLDLLVSFYFQEQGYVANKYLDKKRIEAILPKVSVKSEESLYAAVGFGLDISPISVFNKLTEKER
5 REERAKAKAEAEELVKGGEVKHENKDVLKVRSENGVIIQGASGLLMRIAKCCNPVPGDPIDGYITKGRG
IAIHRSDCHNIKSQDGYQERLIEVEWDLNSSKDYQAEIDIYGLNRSGLLNDVLQILSNSTKSISTVNAQ
PTKDMKFANIHVSFGIPNLTHLTTVVEKIKAVPDVYSVKRTNG

SEQ ID NO: 12

10 ATGAGGAACGAAATGGCAAAAATAATGAACGTAACAGGAGAAGAAGTCATTGCCTTAGCGGCCACCTATA
TGACCAAGGCTGATGTGGCTTTTGTGGCAAAGGCTTTAGCATATGCAACAGCGGCCCATTTCTACCAAGT
GAGAAAGTCAGGCGAACCTATATCGTCCATCCGATTGAGGTGGCGGGGATTCTGGCTGATTGTCATCTG
GATGCTGTGACAGTTGCTTGTGGCTTTTACATGATGTCGTAGAAGATACGGATATTACCTTAGATGAGA
TCGAAGCAGACTTTGGCCATGATGCTCGTGATATCGTTGATGGTGTACCAAGTTAGGTGAAGTTGAGTA
CAAATCTCATGAGGAGCAACTCGCCGAAAACCATCGCAAAATGCTGATGGCTATGTCCAAAGATATTCGC
15 GTGATTTTGGTGAAATTGGCTGACCGCTGCATAATATGCGCACCCTCAAACATTTGCGCAAGGACAAAC
AAGAGCGCATTTCGCGCGAAACCATGGAAATCTATGCCCCCTTGGCGCATCGTTTGGGGATTAGTCGCAT
CAAATGGGAAGTAGAAGATTTGGCTTTTTCGTTACCTCAATGAAACCGAATTTTACAAAATTTCCCATATG
ATGAAAGAAAAACGTCGCGAGCGTGAAGCTTTGGTAGAGGCTATTGTCAGTAAGGTCAAACCTATACGA
CACAACAAGGGTTGTTTGGAGATGTGTATGGCCGACCAAAACACATTTATTCGATTTATCGGAAAATGCG
20 GGACAAAAAGAAACGATTGATCAGATTTTGTATCTGATTGCCATTTCGTTGTGTCATGGAAACGCAAAGC
GATGTCTATGCTATGGTTGGCTATATTCATGAGCTTTGGCGTCCCATGCCAGGCCGCTTCAAGGATTATA
TTGCAGCTCCTAAAGCTAATGGCTACAGTCTATTTCATACCACCGTGTATGGGCCAAAAGGACCTATTGA
GATTCAAATCAGAACTAAGGACATGCATCAAGTGGCTGAGTACGGGGTTGCTGCTCACTGGGCTTATAAA
AAAGGCGTGCCTGTAAGGTCAATCAAGCTGAGCAAGCCGTTGGCATGAACTGGATCAAAGAGCTGGTAG
25 AATTGCAAGATGCCTCAAATGGCGATGCAGTGGACTTTGTGGATTCCGTCAAAGAAGACATTTTTCTGA
ACGGATTTATGTCTTTACACCGACAGGGGCCGTTGAGGAGTTACAAAAGAATCAGGTCTTATTGATTTT
GCTTATGCGATCCATACGCAAATCGGTGAAAAAGCAACAGGTGCCAAAGTCAATGGACGTATGGTTCCTC
TCACTGCCAAGTTAAAAACAGGAGATGTGGTTGAAATCATCACCATGCCAATTCCTTTGGCCCTAGTCG
AGACTGGGTAAACTGGTCAAACCAATAAGGCTCGCAACAAAATTCGTGAGTTCTTTAAAAATCAAGAC
30 AAGGAATTGTCAGTGAATAAAGCCGTTGATTGTTGGTGTCTTATTTTCAAGAGCAGGGCTACGTTGCCA
ATAAATACCTTGACAAAAACGCATTGAAGCCATCCTTCCAAAAGTCAGTGTGAAGAGCGAAGAATCACT
CTATGCAGCCGTTGGGTTTGGTGACATTAGTCCTATCAGTGTCTTAAACAAGTTAACCGAAAAAGAGCGC
CGTGAAGAAGAAAGGGCCAAGGCTAAAGCAGAAGCTGAAGAATTGGTTAAGGGCGGTGAGGTCAAACACG
AAAACAAAGATGTGCTCAAGGTTTCGAGTGAAGATGGAGTCATTATCCAAGGAGCATCAGGCCTCTTGAT
35 GCGGATTGCCAAGTGTGTAATCCTGTACCTGGTGATCCTATTGACGGCTACATTACCAAAGGGCGTGGC
ATTGCGATTACAGATCGGACTGTGATAACATTAAGAGTCAAGATGGCTACCAAGAACGCTTGATTGAGG
TCGAGTGGGATTGGACAATTGAGTAAAGATTATCAGGCTGAAATTGATATCTATGGGCTCAATCGTAG
TGGTCTGCTTAATGATGTGCTCCAAATTTTATCAAACCTCAACCAAGAGCATATCGACAGTCAATGCTCAG
CCGACCAAGGACATGAAGTTTGCTAATATTACGTGAGCTTTGGCATTCCAAATCTGACGCATCTGACCA
40 CTGTTGTGCAAAAAATCAAGGCAGTTCCAGATGTTTATAGCGTGAAGCGGACCAATGGCTAA

Preferred GAS 389 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 11; and/or (b) which is a fragment of at least n
45 consecutive amino acids of SEQ ID NO: 11, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These GAS 389 proteins include variants
(e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 11. Preferred
fragments of (b) comprise an epitope from SEQ ID NO: 11. Other preferred fragments lack one or
more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one
50 or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ
ID NO: 11. Other fragments omit one or more domains of the protein (e.g. omission of a signal
peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(7) GAS 504

GAS 504 corresponds to M1 GenBank accession numbers GI:13622806 and GI:15675600, to M3 GenBank accession number GI: 21911061, to M18 GenBank accession number GI: 19746708, and is also referred to as 'Spy1751' (M1), 'SpyM3_1525', 'SpyM18_1823' (M18) and 'fabK'. GAS 504 has also been identified as a putative trans-2-enoyl-ACP reductase II. Amino acid and polynucleotide sequences of GAS 504 of an M1 strain are set forth below:

SEQ ID NO: 13

MKTRITELNIDYPIFQGGMAWVADGDLGAVSNAGGLGIIGGGNAPKEVVKANIDRVKAITDRPFGVNI
MLLSPFADDIVDLVIEGVKVVTTGAGNPGKYMERLHQAGIIVVPVPSVALAKRMEKLGVDVIAEGME
AGGHIGKLTMSLVQRQVVEAVSIPVIAAGGIADGHGAAAFMLGAEAVQIGTRFVAKESNAHQNFKDKI
LAAKDIDTVISAQVVGHPVRSIKNKLTSAYAKAEKAFLIGQKTATDIEEMGAGSLRHAVIEGDVNGSVM
AGQIAGLVRKEESCETILKDIYYGAARVIQNEAKRWQSVSIEK

SEQ ID NO: 14

ATGAAAACACGTATTACAGAATTACTTAATATTGATTACCCCATTTTTCAAGGAGGAATGGCTTGGGTTG
CTGATGGTGATTTAGCAGGTGCAGTTTCTAATGCTGGTGGTTTAGGCATTATAGGTGGTGGCAATGCTCC
CAAAGAAGTCGTTAAAGCTAATATTGATCGTGTCAAAGCTATTACTGATAGACCTTTTGGGGTTAATATC
ATGCTTTTATCTCCTTTTGTCTGATGATATCGTTGATCTGGTCATTGAAGAAGGTGTTAAAGTAGTAACAA
CAGGCGCAGGAAATCCAGGAAAGTATATGGAAAGACTGCACCAGGCGGGTATAATCGTTGTTCTGTGTG
CCCAAGCGTTGCGCTAGCCAAACGTATGGAAAGCTTGGGGTAGATGCTGTTATTGCTGAGGGTATGGAA
GCTGGAGGACATATTGGCAAGTTAACGACTATGCTTTAGTAAGACAAGTTGTTGAAGCGGTTTCGATTTC
CTGTCAATTGCGGCAGGTGGTATAGCTGATGGTCATGGTGCAGCAGCAGCATTATGTTAGGAGCAGAGGC
TGTTCAAATTGGAACCTCGCTTTGTTGTTGCTAAAGAATCCAATGCTCACCAAAATTTTAAAGATAAAATC
TTAGCAGCAAAAGATATTGATACGGTGATTTCTGCGCAGGTTGTGGGCCACCTGTCCGTTCTATTAAAA
ATAAATTGACCTCAGCTTACGCTAAAGCAGAAAAAGCATTTTTAAATTGGTCAAAAAACAGCTACTGATAT
TGAAGAAATGGGAGCAGGATCGCTTCGACACGCTGTTATTGAAGGCGATGTAGTCAATGGATCTGTTATG
GCTGGCCAAATTGCAGGGCTTGTGAGAAAAGAAGAAAGCTGTGAAACGATTTTAAAGATATTTATTATG
GTGCAGCTCGTGTTATTCAAATGAAGCTAAGCGCTGGCAATCTGTTTCAATAGAAAAGTAG

Preferred GAS 504 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 13, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 504 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 13. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 13. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 13. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(8) GAS 509

GAS 509 corresponds to M1 GenBank accession numbers GI:13622692 and GI:15675496, to M3 GenBank accession number GI: 21910899, to M18 GenBank accession number GI: 19746544, and is also referred to as 'Spy1618' (M1), 'SpyM3_1363' (M3), 'SpyM18_1627' (M18) and 'cysM'. GAS 509 has also been identified as a putative O-acetylserine lyase. Amino acid and polynucleotide sequences of GAS 509 of an M1 strain are set forth below:

SEQ ID NO: 15

MTKIYKTITELVGQTPIIKLNRLIPNEAADVYVKLEAFNPGSSVKDRIALSMIEAABAEGLISPGDVIIE
PTSGNTGIGLAWVGAAGYRVIIIVMPETMSLERRQIIQAYGAELVLTPGAEGMKGAIAKAETLAIELGAW
MPMQFNPNANPSIHEKTTAQEILEAPKEISLDAFVSGVGTGGTLSGVSHVLKKNPETHVIYAVBAESSAV
5 LSGQEPGPHKIQGISAGFIPTNLDTKAYDQIIIRVKSDALETARLTGAKEGFLVGISSGAALYAAIEVAK
OLGKKGKHLTILPDNGERYLSTELYDVPVIKTK

SEQ ID NO: 16

ATGACTAAAATTTACAAAACCTATAACAGAATTAGTAGGTCAAACACCTATTATCAAACCTTAACCGTTTAA
10 TTCCAAACGAAGCTGCTGACGTTTATGTAAAATTAGAAGCTTTTAACCCAGGATCTTCTGTAAAGATCG
TATTGCTTTATCGATGATTGAAGCTGCTGAAGCTGAAGGTCTGATAAGTCTGGTGACGTTATTATCGAA
CCAACAAGTGGTAATACAGGTATTGGTCTTGCATGGGTAGGTGCTGCTAAAGGGTATCGAGTCATTATTG
TTATGCCCCGAAACTATGAGCTTGGAAGACGGCAAATCATTAGGCTTATGGTGCAGAGCTTGTCTTAAC
ACCTGGAGCAGAAGGTATGAAAGGGGCTATTGCAAAGCTGAACTTTAGCAATAGAACTAGGTGCTTGG
15 ATGCCTATGCAATTTAATAACCTGCCAATCCAAGCATCCATGAAAAACAACAGCTCAAGAAATTTTGG
AAGCTTTTAAGGAGATTTCTTTAGATGCATTCGTATCTGGTGTGGTACTGGAGGAACACTTTCTGGTGT
TTCACATGTCTTGAAAAAGCTAACCTGAACTGTTATCTATGCTGTTGAAGCTGAAGAATCTGCTGTC
TTATCTGGTCAAGAGCCTGGACCACATAAAATTCAAGGTATATCAGCTGGATTTATCCAAACACGTTAG
ATACCAAAGCCTATGACCAAATTATCCGTGTTAAATCGAAAGATGCTTTAGAACTGCTCGACTAACAGG
20 AGCTAAGGAAGGCTTCCTGGTTGGGATTTCTTCTGGAGCTGCTCTTTACGCCGCTATTGAAGTCGCTAAA
CAGTTAGGAAAAGGCAAACATGTGTTAACTATTTACCAGATAATGGCGAACGCTATTTATCGACTGAAC
TCTATGATGTACCAGTAATTAAGACGAAATAA

Preferred GAS 509 proteins for use with the invention comprise an amino acid sequence: (a) having
25 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 15; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 15, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 509 proteins include variants (e.g. allelic
variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 15. Preferred fragments of (b)
30 comprise an epitope from SEQ ID NO: 15. Other preferred fragments lack one or more amino acids
(e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 15. For
example, in one embodiment, the underlined amino acid sequence at the C-terminus of SEQ ID NO:
15 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a signal
35 peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(9) GAS 366

GAS 366 corresponds to M1 GenBank accession numbers GI:13622612, GI:15675424 and
GI:30315979, to M3 GenBank accession number GI: 21910712, to M18 GenBank accession number
GI: 19746474, and is also referred to as 'Spy1525' (M1), 'SpyM3_1176' (M3), 'SpyM18_1542'
40 (M18) and 'murD'. GAS 366 has also been identified as a UDP-N-acetylmuramoylalanine-D-
glutamate ligase or a D-glutamic acid adding enzyme. Amino acid and polynucleotide sequences of
GAS 366 of an M1 strain are set forth below:

SEQ ID NO: 17

MKVISNFQNKKILILGLAKSGEAAKLLTKLGALVTVNDSPKFDQNPAAQALLBEGIKVICGSHPVVELLD
45 ENFEYMKVKNPGIPYDNPVMVKRALAKEIPILTEVELAYFVSEAPIIGITGSNGKTTTTMIADVLNAGGQS
ALLSGNIGYPASKVVQKAIAGDTLVMELSSFLVGVNAFRPHIAVITNLMPTHLDYHGSFEDYVAAKWTI
QAQMTESDYLIINANQBSATLAKTTKATVIPFSTQKVVDGAYLKDGILYFKEQAI AATDLGVPGSHNI
ENALATIAVAKLSGIADDIIAQCLSHFGVKHRLQRVGQIKDITFYNSKSTNIIATQKALSGFDNSRLI
LIAGGLDRGNEFDLVPDLLGLKQMIILGESAERMKRAANKAEVSYLEARNVAEATELAFKLAQTGDTIL

LSPANASWDMYPNFEVRGDEFLATFDCLRGDA

SEQ ID.NO: 18

ATGAAAGTGATAAGTAATTTTCAAAAACAAAAAATATTAATATTGGGGTTAGCCAAATCGGGCGAAGCAG
CAGCAAAATTATTGACCAAACTTGGTGCTTTAGTGACTGTTAATGATAGTAAACCATTTGACCAAAATCC
5 AGCGGCACAAGCCTTGTTGGAAGAGGGGATTAAGGTCATTTGTGGTAGCCACCCAGTAGAATTATTAGAT
GAGAACTTTGAGTACATGGTTAAAAACCTGGGATTCCCTTATGATAATCCTATGGTTAAACGCGCCCTTG
CAAAGGAAATTCCCATCTTGACTGAAGTAGAATTGGCTTATTTCTGATCTGAAGCGCCTATTATCGGGAT
TACAGGATCAAACGGGAAGACAACCACAACGACAATGATTGCCGATGTTTGAATGCTGGCGGGCAATCT
GCACTCTTATCTGGAAACATTGGTTATCCTGCTTCAAAAGTTGTTCAAAAAGCAATTGCTGGTGATACTT
10 TGGTGATGGAATTGTCCTCTTTTCAATTAGTGGGAGTGAATGCTTTTCGCCCTCATATTGCTGTCATCAC
TAATTTAATGCCGACTCACCTGGACTATCATGGCAGTTTGGAGATTATGTTGCTGCTAAATGGATGATT
CAAGCTCAGATGACAGAATCAGACTACCTTATTTTAAATGCTAATCAAGAGATTTCAGCAACTCTAGCTA
AGACCACCAAAGCAACAGTGATTCCCTTTTCAACTCAAAAAGTGGTTGATGGAGCTTATCTGAAGGATGG
AATACTCTATTTTAAAGAACAGGCGATTATAGCTGCAACTGACTTAGGTGTCCAGGTAGCCACAACATT
15 GAAATGCCCTAGCAACTATTGCAGTTGCCAAGTTATCTGGTATTGCTGATGATATTATTGCCAGTGCC
TTTCACATTTTGGAGGCGTTAAACATCGTTTGCAACGGGTTGGTCAAATCAAAGATATTACCTTCTACAA
TGACAGTAAGTCAACCAATATTTTAGCCACTCAAAAAGCTTTATCAGGTTTGGATAACAGTCGCTTGATT
TTGATTGCTGGCGGTCTAGATCGTGGAATGAATTTGACGATTTGGTGCCAGACCTTTTAGGACTTAAGC
AGATGATTATTTTGGGAGAATCCGCAGAGCGTATGAAGCGAGCTGCTAACAAAGCAGAGGTCTCTTATCT
20 TGAAGCTAGAAATGTGGCAGAAGCAACAGAGCTTGCTTTTAAAGCTGGCCCAAACAGGCGATACTATCTTG
CTTAGCCAGCCAATGCTAGCTGGGATATGTATCCTAATTTTGAAGTTCTGGGGATGAATTTTGGCAA
CCTTTGATTGTTTAAAGAGGAGATGCCTAA

Preferred GAS 366 proteins for use with the invention comprise an amino acid sequence: (a) having
25 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 17; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 17, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 366 proteins include variants (e.g. allelic
variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 17. Preferred fragments of (b)
30 comprise an epitope from SEQ ID NO: 17. Other preferred fragments lack one or more amino acids
(e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 17. For
example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO:
17 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a signal
35 peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(10) GAS 159

GAS 159 corresponds to M1 GenBank accession numbers GI:13622244 and GI:15675088, to M3
GenBank accession number GI: 21910303, to M18 GenBank accession number GI: 19746056, and is
also referred to as 'Spy1105' (M1), 'SpyM3_0767' (M3), 'SpyM18_1067' (M18) and 'potD'. GAS
40 159 has also been identified as a putative spermidine/putrescine ABC transporter (a periplasmic
transport protein). Amino acid and polynucleotide sequences of GAS 159 of an M1 strain are set
forth below:

SEQ ID NO: 19

MRKLYSFLAGVLGVIVILTSLSFILQKKSQSGSQSDKLVIYNWGDYIDPALLKKFTKETGIEVQYETFDS
45 NEAMYTKIKQGGTTYDIAVPSDYTDKMIKENLLNKLDKSKLVGMDNIGKEFLGKSFPDQNDYSLPYFWG
TVGIVYNDQLVDKAPMHWEDLWRPEYKNSIMLIDGAREMLGVGLTTFGYSVNSKNLEQLQAAERKLOQLT
PNVKAIVADEMKGYMIQGDAAIGITPSGEASEMLDSNEHLHYIVPSEGSNLWFDNLVLPKTMKHEKBAYA
FLNFINRPENAAQNAAYIGYATPNKKAKALLPDEIKNDPAFYPTDDI IKKLEVDNLGSRWLGIYNDLYL

QFKMYRK

SEQ ID NO: 20

5 ATGCGTAAACTTTATTCCTTTCTAGCAGGAGTTTTGGGTGTTATTGTTATTTTAACAAGTCTTTCTTTCA
TCTTGCAGAAAAATCGGGTTCTGGTAGTCAATCGGATAAATTAGTTATTTATAACTGGGGAGATTACAT
TGATCCAGCTTTGCTCAAAAAATTCACCAAAGAAACGGGCATTGAAGTGCAGTATGAAACTTTTCGATTCC
AATGAAGCCATGTACACTAAAATCAAGCAGGGCGGAACCACTTACGACATTGCTGTTCTTAGTGATTACA
CCATTGATAAAATGATCAAAGAAAACCTACTCAATAAGCTTGATAAGTCAAAATTAGTTGGCATGGATAA
10 TATCGGGAAAGAATTTTATAGGGAAAAGCTTTGACCCACAAAACGACTATTCTTTGCCTTATTTCTGGGGA
ACCGTTGGGATTGTTTATAATGATCAATTAGTTGATAAGGCGCCTATGCACTGGGAAGATCTGTGGCGTC
CAGAATATAAAAAATAGTATTATGCTGATTGATGGAGCGCGTGAATGCTAGGGGTTGGTTTAAACAACTTT
TGGTTATAGTGTGAATTCTAAAAATCTAGAGCAGTTGCAGGCAGCCGAGAGAAAACCTGCAGCAGTTGACG
CCGAATGTTAAAGCCATTGTAGCAGATGAGATGAAAGGCTACATGATTCAAGGTGACGCTGCTATTGGAA
TTACCTTTTCTGGTGAAGCCAGTGAGATGTTAGATAGTAACGAACACCTTCACTACATCGTGCCTTCAGA
15 AGGGTCTAACCTTTGGTTTGATAATTTGGTACTACCAAAAACCATGAAACACGAAAAAGAAGCTTATGCT
TTTTTGAACCTTATCAATCGTCTGAAAATGCTGCGCAAAATGCTGCATATATTGGTTATGCGACACCAA
ATAAAAAAGCCAAGGCCCTTACTTCCAGATGAGATAAAAAATGATCCTGCTTTTTATCCAACAGATGACAT
TATCAAAAAATTGGAAGTTTATGACAATTTAGGGTCAAGATGGTTGGGGATTATAATGATTTATACCTC
CAATTTAAATGTATCGCAAATAA

20 Preferred GAS 159 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 19; and/or (b) which is a fragment of at least *n*
consecutive amino acids of SEQ ID NO: 19, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
25 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS 159 proteins include variants (e.g. allelic
variants, homologs, orthologs, paralog, mutants, etc.) of SEQ ID NO: 19. Preferred fragments of (b)
comprise an epitope from SEQ ID NO: 19. Other preferred fragments lack one or more amino acids
(e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 19. For
30 example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO:
19 is removed. In another example, the underlined amino acid sequence at the C-terminus of SEQ ID
NO: 19 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a
signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(11) GAS 217

35 GAS 217 corresponds to M1 GenBank accession numbers GI:13622089 and GI:15674945, to M3
GenBank accession number GI: 21910174, to M18 GenBank accession number GI: 19745987, and is
also referred to as 'Spy0925' (M1), 'SpyM3_0638' (M3), and 'SpyM18_0982' (M18). GAS 217 has
also been identified as a putative oxidoreductase. Amino acid and polynucleotide sequences of GAS
217 of an M1 strain are set forth below:

SEQ ID NO: 21

40 MAQRIVITGASGGLAQAI VKQLPKEDSLILLGRNKERLEHCYQHIDNKECLELDITNPVAIEKMVAQIY
QRYGRIDVLINNAGYGAFKGFEEFSAQEIADMFOVNTLASIHFACLIGQKMAEQGQGH LINIVSMAGLIA
SAKSSIYSATKFALIGFSNALRLBLADKGVYVTTVNP GPIATKFFDQADPSGHYLESVGKFTLQPNQVAK
45 RLVSIIGKNKRELNLPFLAVTHQFYTLFPKLS DY LARKVFNYK

SEQ ID NO: 22

ATGGCACAAGAATCATTGTTATCACGGGAGCTTCTGGAGGACTGGCTCAGGCAATTGTTAAGCAGTTAC
CCAAGGAAGACAGCTTGATTTTATTAGGACGTAACAAAGAACGCCTAGAACACTGTTATCAGCATATTGA

CAACAAAGAATGCCTCGAGTTGGATATTACCAATCCAGTAGCCATTGAGAAAATGGTCGCCCAGATTAC
CAGCGCTATGGCCGTATTGATGTCTTGATTAATAATGCTGGCTACGGAGCTTTCAAAGGCTTTGAAGAGT
TTTCTGCCCAAGAAATAGCTGATATGTTTCAGGTTAACACCCTAGCGAGCATTCACTTTGCTTGCTTGAT
TGGTCAGAAAATGGCAGAGCAGGGGCAAGGTCACCTTATTAATATTGTGTCCATGGCAGGCTTGATTGCG
5 TCAGCCAAATCGAGCATTATTCAGCCACCAAGTTTGCCCTTATCGGATTTTCCAATGCCCTTCGCTTAG
AATTAGCGGATAAAGGGGTTTACGTGACCACCGTGAATCCAGGTCCCATTGCCACCAAGTTTTTTGACCA
AGCTGACCCGTCTGGACATTATTTGGAAAGCGTTGGTAAATTTACTCTCCAACCAATCAAGTGGCTAAG
CGTTTGGTTTCTATTATCGGGAAAAATAAACGAGAATTGAATTTGCCCTTAGTTTAGCGGTGACCCATC
AATTTTACACCCTTTTCCCTAAATTATCTGATTATCTTGCAAGAAAGGTATTTAATTATAAATGA

10 Preferred GAS 217 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 21; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 21, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
15 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 217 proteins include variants (e.g. allelic
variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 21. Preferred fragments of (b)
comprise an epitope from SEQ ID NO: 21. Other preferred fragments lack one or more amino acids
(e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 21.
20 Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a
cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(12) GAS 309

GAS 309 corresponds to M1 GenBank accession numbers GI:13621426 and GI:15674341, to M3
GenBank accession number GI: 21909633, to M18 GenBank accession number GI: 19745363, and is
25 also referred to as 'Spy0124' (M1), 'SpyM3_0097' (M3), 'SpyM18_0205' (M18), 'nra' and 'rofA'.
GAS 309 has also been identified as a regulatory protein and a negative transcriptional regulator.
Amino acid and polynucleotide sequences of GAS 309 of an M1 strain are set forth below:

SEQ ID NO: 23

30 MIEKYLESSIESKQQLIVLFFPKTSYLPITEVAEKTGLTFLQLNHYCEELNAFFPGSLSMITQKRMISCQF
THPFKETYLYQLYASSNVLQLLAFLIKNGSHSRPLTDFARSHFLSNSSAYRMREALIPLLRNFBKLKSKN
KIVGEEYRIRYLIALLYSKFGIKVYDLTQQDKNTIHSFLSHSSTHLKTSPLWSESFYDILLALSWKRH
QFSVTIPQTRIFQQLKLFVYDSLKKSSHDI IETYCQLNFSAGDL DYLYLIYITANNSFASLQWTPEHIR
QYCQLFEENDTFRLLLNPIITLLPNLKEQKASLVKALMFFSKSFLNLFHFI PETNLFVSPYYKGNQKLY
TSLKLIVEEWMKLPGRDLNKH FHLFCHYVEQSLRNIQPPLVVVFVASFNAHLLTDSFPRYFSDKS
35 IDFHSYLLQDNVYQIPDLKPDLVITHSQLIPFVHHELTGKIAVAEISFDESILSIQELMYQVKEEFQA
DLTKQLT

SEQ ID NO: 24

40 TTGATAGAAAAATACTTGGAATCATCAATCGAATCAAAATGTCAGTTAATTGCTTGTTTTTTAAGACAT
CTTATTTGCCAATAACTGAGGTAGCAGAAAAAAGTGGCTTAACCTTTTACAATAAACCATTATTGTGA
GGAAGTGAATGCCTTTTCCCTGGTAGTCTGTCTATGACCATCAAAAAAGGATGATATCTTGCCAATTT
ACACATCCTTTTAAAGAACTTATCTTTACCAACTCTATGCATCATCTAATGTCTTACAATTACTAGCCT
TTTTAATAAAAAATGGTTCCCACTCTCGTCCCCCTTACGGATTTTGCAAGAAGTCATTTTATCAAATC
CTCAGCTTATCGGATGCGGAAGCATTGATTCTTTATTAAGAACTTTGAATTAAACTCTCTAAGAAC
45 AAGATTGTCGGTGAGGAATATCGCATCCGTTACCTCATCGCTCTGCTATATAGTAAGTTTGGCATTAAAG
TTTATGACTTGACGCAGCAAGACAAAAACACTATTCATAGCTTTTATCCCATAGTTCCACCCACCTTAA
AACCTCTCCTTGGTTATCGGAATCGTTTCTTTCTATGACATTTTATTAGCTTTATCGTGGAAGCGGCAT
CAATTTTCGGTAAGTATTTCCCAAACCAGAAATTTTCAACAATTAAAAAACTTTTGTCTACGATTCTT
TGAAAAAAGTAGCCATGATATTATCGAACTTACTGCCAACTAACTTTTTCAGCAGGAGATTGGACTA
50 CCTCTATTTAATTTATATCACCGCTAATAATTCTTTTTCGAGCTTACAATGGACACCTGAGCATATCAGA

CAATATTGTCAACTTTTGAAGAAAATGATACTTTTCGCCTGCTTTTAAATCCTATCATCACTCTTTTAC
CTAACCTAAAAGAGCAAAAGGCTAGTTTAGTAAAAGCTCTTATGTTTTTTTCAAATCATTCTTGTTTAA
TCTGCAACATTTTATTCCTGAGACCAACTTATTCGTTTTCTCCGTACTATAAAGGAAACCAAAACTCTAT
ACGTCCTTAAAGTTAATTGTCGAAGAGTGGATGGCCAAACTTCCTGGTAAGCGTGACTTGAACCATAAGC
5 ATTTTCATCTTTTTTGCCACTATGTCGAGCAAAGTCTAAGAAATATCCAACCTCCTTTAGTTGTTGTTTT
CGTAGCCAGTAATTTTATCAATGCTCATCTCCTAACGGATTCTTTTCCAAGGTATTTCTCGGATAAAAGC
ATTGATTTTCATTCTTATTATCTATTGCAAGATAATGTTTATCAAATTCCTGATTTAAAGCCAGATTG
TCATCACTCACAGTCAACTGATTCCTTTTGTTCACCATGAACCTTACAAAAGGAATTGCTGTTGCTGAAAT
ATCTTTTGATGAATCGATTCTGTCTATCCAAGAATTGATGTATCAAGTTAAAGAGGAAAAATTCCAAGCT
10 GATTTAACCAAGCAATTAACATAA

Preferred GAS 309 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 23; and/or (b) which is a fragment of at least n
15 consecutive amino acids of SEQ ID NO: 23, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These GAS 309 proteins include variants (e.g. allelic
variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 23. Preferred fragments of (b)
comprise an epitope from SEQ ID NO: 23. Other preferred fragments lack one or more amino acids
(e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino
20 acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 23.
Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a
cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(13) GAS 372

GAS 372 corresponds to M1 GenBank accession numbers GI:13622698 and GI:15675501, to M3
25 GenBank accession number GI: 21910905, to M18 GenBank accession number GI: 19746500 and is
also referred to as 'Spy1625' (M1), 'SpyM3_1369' (M3), and 'SpyM18_1634' (M18). GAS 372 has
also been identified as a putative protein kinase or a putative eukaryotic-type serine/threonine kinase.
Amino acid and polynucleotide sequences of GAS 372 of an M1 strain are set forth below:

SEQ ID NO: 25

30 MIQIGKLFAGRYRILKSIGRGGMADVYLANDLILDNEDVAIKVLRNTNYQTDQVAVARFQREARAMAELNH
PNIVAIRDIGEEDGQQLVMEYVDGADLKRYIQNHAPLSNNEVVRIMEEVLSAMTLAHQKGI VHRDLKPQ
NILLTKEGVVKVTDGFI AVAFAETSLTQTNSMLGSVHYLSPEQARGSKATI QSDIYAMGIMLFEMLTGHI
PYDGDSAVTIALQHFKPLPSII EENHNVPQALENVVIRATAKKLSDRYGSTFEMSRDLMTALSYNRSRE
RKIIIFENVESTKPLPKVASGPTASVKLSPPPTPTVLTQESRLDQTNQTDALQPPTKKKSGRFLGTLFKIL
35 FSPFIVGVALFTYLILTKPTSVKVPNVAGTSLKVAKQELYDVGLKVGKIRQIESDTVAEGNVVVRTDPKAG
TAKRQGSSITLYVSIGNKGFDMENYKGLDYQEAMNSLIETYGVPKSKI KIERIVTNEY PENTVISQSPSA
GDKFNPNGKSKITLSVAVSDTITMPMVTEYSYADAVNTLTALGIDASRIKAYVPSSSSATGFVPIHSPSS
KAIVSGQSPYYGTSLSLSDKGEISLYLYPEETHSSSSSSSSSTSSSNSSSINDSTAPGSNTELSPSETTSQ
40 TP

SEQ ID NO: 26

ATGATTCAGATTGGCAAATTATTTGCTGCTCGTTATCGCATTCCTGAAATCTATTGGCCGCGGTGGTATGG
CGGATGTTTATTAGCAAATGACTTGATCTTGATAATGAAGACGTTGCAATCAAGGTCTTGCGTACCAA
TTATCAAACAGATCAGGTAGCAGTTGCGCGTTTCCAACGAGAAGCGCGGGCCATGGCTGAATTGAACCAT
45 CCCAATATTGTTGCCATCCGGGATATAGGTGAAGAAGACGGACAGCAATTTTATGTAATGGAATATGTGG
ATGGTGCTGACCTAAAGAGATACATTCAAATCATGCTCCATTATCTAATAATGAAGTGGTTAGAATTAT
GGAAGAAGTCCTTTCTGCTATGACTTTAGCCCACCAAAAAGGAATTGTACACAGAGATTTAAAACCTCAA
AATATCCTACTAATAAGGAGGGTGTGTCAAAGTAACTGATTTCCGCATCGCAGTAGCCTTTGCAGAAA
CAAGCTTGACACAACTAATTGATGTTAGGCAGTGTTCACTTGTCTCCAGAACAGGCTCGCGGCTC
50 CAAAGCGACGATTCAAAGTGATTTTATGCGATGGGGATTATGCTCTTTGAGATGTTGACAGGCCATATC

CCTTATGACGGCGATAGTGCTGTTACGATTGCCTTGCAACATTTTCAAAGCCTCTTCCATCTATTATCG
AGGAGAACCACAATGTGCCACAAGCTTTGGAGAATGTTGTTATTCGAGCAACAGCCAAGAAATTAAGTGA
TCGTTACGGGTCAACCTTTGAAATGAGTCGTGACTTAATGACGGCGCTTAGTTATAATCGTAGTCGGGAG
CGTAAGATTATCTTTGAGAATGTTGAAAGTACCAAACCCCTCCCCAAAGTGGCCTCAGGTCCCACCGCTT
5 CTGTAAAATTGTCTCCCCCTACCCCAACAGTGTTAACACAGGAAAGTCGATTAGATCAAATAATCAAAC
AGATGCTTTACAGCCCCCCCACCAAAAAGAAAAAAGTGGTCGTTTTTTAGGTACTTTATTCAAATTTCTT
TTTTCTTTCTTTATTGTAGGTGTAGCACTCTTTACTTATCTTATACTAACTAAACCAACTTCTGTGAAAG
TTCCTAATGTAGCAGGCACTAGTCTTAAAGTTGCCAAACAAGAACTGTATGATGTTGGGCTAAAAGTGGG
TAAAATCAGGCAAATTGAGAGTGATACGGTTGCTGAGGGAAATGTAGTTAGAACAGATCCTAAAGCAGGA
10 ACAGCTAAGAGGCAAGGCTCAAGCATTACGCTTTATGTGTCAATTGGAAACAAAGGTTTTGACATGGAAA
ACTACAAAGGACTAGATTATCAAGAAGCTATGAATAGTTTGATAGAACTTATGGTGTTCCAAATCAAA
AATCAAATTTGAGCGCATTGTAATAATGAATATCCTGAAAATACAGTCATCAGTCAATCGCCAAGTGCG
GGTGATAAATTTAATCCAAACGGAAGTCTAAAATTACGCTCAGTGTGCTGTTAGTGATACGATCACTA
TGCCTATGGTAACAGAATATAGTTATGCAGATGCAGTCAATACCTTAACAGCTTTAGGTATAGATGCATC
15 TAGAATAAAAGCTTATGTGCCAAGCTCTAGCTCAGCAACGGGCTTTGTGCCAATTCATTCTCCTAGTTCT
AAAGCTATTGTGAGTGGTCAATCTCCTTACTATGGAACGTCTTTGAGTCTGTCTGATAAAGGAGAGATTA
GTCTTTACCTTTATCCAGAAGAAACACACTCTTCTAGTAGCTCATCGAGTTCAACGTCAAGTTCAAACAG
TTCTTCAATAAATGATAGTACTGCACCAGGTAGCAACACTGAATTAAGCCCATCAGAACTACTTCTCAA
ACACCTTAA

20 Preferred GAS 372 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 25; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 25, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
25 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These GAS 372 proteins include variants
(e.g. allelic variants, homologs, orthologs, paralog, mutants, etc.) of SEQ ID NO: 25. Preferred
fragments of (b) comprise an epitope from SEQ ID NO: 25. Other preferred fragments lack one or
more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one
or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ
30 ID NO: 25. Other fragments omit one or more domains of the protein (e.g. omission of a signal
peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(14) GAS 039

GAS 039 corresponds to M1 GenBank accession numbers GI:13621542 and GI:15674446, to M3
GenBank accession number GI: 21909730, to M18 GenBank accession number GI: 19745398 and is
35 also referred to as 'Spy0266' (M1), 'SpyM3_0194' (M3), and 'SpyM18_0250' (M18). Amino acid
and polynucleotide sequences of GAS 039 of an M1 strain are set forth below:

SEQ ID NO: 27

MDLILFLLVLVLLGLGAYLLFKVNGLQHQLAQTLGNADNLSQDMTYQLDTANKQQLLELTQLMNRQQAG
40 LYQQLTDIRDVLHRSLSRDRSDKRLEKINQQVNQSLKNMQESNEKRLEKMRQIVEEKLEETLKNRLHA
SFDSVSKQLESVNKGLGEMRSVAQDVGTLNKLVSNTKTRGILGELQLGQIIEDIMTSSQYEREFVTVSGS
SERVEYAIKLPNGQGQGYIYLPIDSKFPLEDYRLEDAYEVGDKLAI EASRKALLAAIKRFAKDIHKKYL
NPPETTNFGVMFLPTEGLYSEVVRNASFFDSLRRREENIVVAGPSTLSALLNSLSVGFKTLNIQKNADDIS
45 KILGNVKLEFDKFGGLLAKAQKQMNTANNTLDQLISTRNIAIVRALNTVETYQDQATKSLNMPLEEN
NEN

SEQ ID NO: 28

ATGGACCTTATCTTGTTCCTTTTGGTCTTGGTTCTCTTAGGTTTAGGGGCTTATCTGTTGTTCAAAGTCA
50 ACGGCCTTCAACATCAGCTTGCCCAAACCCCTAGAAGGCAACGCGGATAATTTGTCTGACCAAATGACCTA
CCAGTTGGATACAGCTAACAAACAACAATTGTTAGAGCTAACACAGCTGATGAACCGACAACAAGCAGGC
CTTTACCAACAATTAACAGATATTCGTGACGTCTTGACCGTAGTTTGTCTGATAGTAGGGACCGGTCTG

ACAAACGCTTAGAAAAAATTAACCAGCAGGTCAACCAATCGCTCAAAAATATGCAAGAATCTAACGAAAA
ACGTTTGGAGAAAATGCGCCAGATCGTTGAAGAAAAATTTGGAAGAAACCTTAAAAAATCGTCTGCACGCC
TCTTTCGATTCTGTATCCAAGCAACTAGAAAGTGTCAATAAAGGCTTGGGAGAAATGCGTAGCGTGGCTC
AAGATGTGGGTACTTTAAATAAGGTTTTGTCCAATACCAAAACACGAGGCATTTTAGGCGAACTTCAACT
5 AGGCCAAATCATTGAGGATATCATGACATCAAGCCAGTACGAAAGAGAATTTGTAACGGTTAGTGGTTCT
AGTGAACCGGTAGAATATGCGATTAAAGCTCCCAGGAAATGGTCAAGGCGGTTATATTTACCTACCGATTG
ACTCAAAATTCCTCTTGAAGATTATTACCGATTAGAAGATGCTTACGAAGTTGGTGATAAACTGGCCAT
CGAGGCTAGCCGAAAAGCACTTCTGGCAGCTATCAAACGCTTTGCCAAAGACATTCATAAAAAGTACTTG
AACCCCCAGAGACGACCAATTTCCGAGTTATGTTCTTACCAACAGAAGGTCTTTATTCAGAAGTGGTCA
10 GAAATGCGTCTTTCTTTGATAGCCTTCGTCCGGAAGAAAATATTGTGGTTGCAGGCCCTTCGACCCTGTC
TGCTTTGCTGAATTCCTTATCTGTTGGTTTCAAGACCTTAATATCCAAAAAATGCTGATGACATCAGT
AAAATTTTAGGCAATGTCAAGTTAGAATTCGATAAAATTTGGCGGCCTGCTTGCCAAGGCTCAAAAACAAA
TGAATACAGCTAATAATACGCTGGATCAGCTCATTTCAACAAGGACAAATGCCATTGTTTCGAGCCTTGAA
TACCGTTGAACTTATCAAGACCAAGCAACAAAATCTCTCTTGAACATGCCCTTATTAGAAGAGGAAAAT
15 AATGAAAATTAA

Preferred GAS 039 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 27; and/or (b) which is a fragment of at least n
20 consecutive amino acids of SEQ ID NO: 27, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 039 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 27. Preferred fragments
of (b) comprise an epitope from SEQ ID NO: 27. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
25 amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
NO: 27. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide,
of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(15) GAS 042

GAS 042 corresponds to M1 GenBank accession numbers GI:13621559 and GI:15674461, to M3
30 GenBank accession number GI: 21909745, to M18 GenBank accession number GI: 19745415, and is
also referred to as 'Spy0287' (M1), 'SpyM3_0209' (M3), and 'SpyM18_0275' (M18). Amino acid
and polynucleotide sequences of GAS 042 of an M1 strain are set forth below:

SEQ ID NO: 29

MTKEKLVAFSQAHAEPWLQERRLAALAEI PNLELPTIERVKFHRWNLGDGTLTENESLASVPDFIAIGD
35 NPKLVQVGTQTVLEQLPMALIDKGVVFSDFYTALEBIPEVIEAHFGQALAFDEDKLAAYHTAYFNAAVL
YVPDHLEITTPIEAIFLQDSDSVPFNKHVLVIAGKESKFTYLERFESIGNATQKISANISVEVIAQAGS
QIKFSAIDRLGPSVTYISRRGRLEKDANIDWALAVMNEGNVIADFDSDLIGQGSQADLKVVAASSGRQV
QGIDTRVTNYGQRTVGHILQHGVLERGTLTENGIGHILKDAKGADAQQESRVLMLSDQARADANPILLI
40 DENEVTAGHAASIGQVDPEDMYILMSRGLDQETAERLVIRGFLGAVIAEIPISVRQEI IKVLDEKLLNR

SEQ ID NO: 30

ATGACAAAAGAAAACTAGTGGCTTTTTTCGCAAGCCCACGCTGAGCCTGCTTGGCTGCAAGAACGGCGTT
TAGCGGCATTAGAAGCCATTCCAAATTTGGAATTACCAACCATCGAAAGGGTTAAATTTACCGTTGGAA
TCTAGGAGATGGTACCTTAACAGAAAATGAAAGTCTAGCTAGTGTTCAGATTTTATAGCTATTGGAGAT
45 AACCCAAAGCTTGTTTCAGGTAGGCACGCAACAGTCTTAGAACAGTTACCAATGGCGTTAATTGACAAGG
GAGTTGTTTTTCAGTGATTTTTATACGGCGCTTGAGGAAATCCCAGAAGTAATTGAAGCTCATTTTGGTCA
GGCATTAGCTTTTGATGAAGACAACTAGCTGCCTACCACACTGCTTATTTTAATAGCGCAGCCGTGCTC
TACGTTCTGATCACTTGGAATCACAACCTCTATTGAAGCTATTTTCTTACAAGATAGTGACAGTGACG
TTCTTTTAAACAAGCATGTTCTAGTGATTGCAGGAAAAGAAAGTAAGTTACCTATTAGAGCGTTTGA
50 ATCTATTGGCAATGCCACTCAAAAGATCAGCGCTAATATCAGTGTAAGAGTATTGCTCAAGCAGGCAGC
CAGATTAAATCTCGGCTATCGACCGCTTAGGTCTTCACTGACAACCTATATTAGCCGTCGAGGACGTT

TAGAGAAGGATGCCAACATTGATTGGGCCTTAGCTGTGATGAATGAAGGCAATGTCATTGCTGATTTTGA
CAGTGATTTGATTGGTCAGGGCTCACAAGCTGATTTGAAAGTTGTTGCAGCCTCAAGTGGTCGTCAGGTA
CAAGGTATTGACACGCGCGTGACCAACTATGGTCAACGTACGGTCGGTCATATTTTACAGCATGGTGTGA
TTTTGGAACGTGGCACCTTAACGTTTAACGGGATTGGTCATATTCTAAAAGACGCTAAGGGAGCTGATGC
5 TCAACAAGAAAGCCGTGTTTTGATGCTTTCTGACCAAGCAAGAGCCGATGCCAATCCAATCCTCTTAATT
GATGAAAATGAAGTAACAGCAGGTCATGCAGCTTCTATCGGTCAGGTTGACCCTGAAGATATGTATTACT
TGATGAGTCGAGGACTGGATCAAGAAACAGCAGAACGATTGGTTATTAGAGGATTCCTAGGAGCGGTTAT
CGCTGAAATTCCTATTCCATCAGTCCGCCAAGAGATTATTAAGGTTTTAGATGAGAAATTGCTTAATCGT
TAA

10 Preferred GAS 042 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 29; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 29, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
15 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 042 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 29. Preferred fragments
of (b) comprise an epitope from SEQ ID NO: 29. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
20 NO: 29. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide,
of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(16) GAS 058

GAS 058 corresponds to M1 GenBank accession numbers GI:13621663 and GI:15674556, to M3
GenBank accession number GI: 21909841, to M18 GenBank accession number GI: 19745567 and is
25 also referred to as 'Spy0430' (M1), 'SpyM3_0305' (M3), and 'SpyM18_0477' (M18). Amino acid
and polynucleotide sequences of GAS 058 of an M1 strain are set forth below:

SEQ ID NO: 31

MKWSGFMKTKSKRFLNLATLCLALLGTTLLMAHPVQAEVISKRDYMTFRGLGDLEDDSANYPNLEARYK
GYLEGYEKGLKGDDI PERPKIQVPEDVQPSDHGDYRDGYEEGFEGEQHKRDPLETEAEDDSQGGRQEGRQ
30 GHQEGADSSDLNVEESDGLSVIDEVVGVIYQAFSTIWTYLSGLF

SEQ ID NO: 32

ATGAAATGGAGTGGTTTTATGAAAACAAAATCAAACGCTTTTTAAACCTAGCAACCCTTTGCTTGGCCC
TACTAGGAACAACCTTGCTAATGGCACATCCCGTACAGGCGGAGGTGATATCAAAAAGAGACTATATGAC
35 TCGCTTCGGGTTAGGCGATTTAGAAGATGATTCAGCTAACTATCCTTCAAATTTAGAAGCTAGATATAAA
GGATATTTAGAGGGATATGAAAAGGCTTAAAGGAGATGATATACCCGAACGGCCCAAGATTCAGGTTC
CTGAGGATGTTTACCCATCTGACCATGGCGACTATAGAGATGGTTATGAGGAAGGATTTGGAGAAGGACA
ACATAAACGTGATCCATTAGAAACAGAAGCAGAAGATGATTCTCAAGGAGGACGTCAAGAAGGACGTCAA
GGACATCAAGAAGGAGCAGATTCTAGTGATTGAACGTTGAAGAAAGCGACGGTTTGTCTGTTATTGATG
40 AAGTAGTTGGAGTAATTTATCAAGCATTAGTACTATTTGGACATACTTAAGCGGTTTGTCTCTAA

Preferred GAS 058 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 31; and/or (b) which is a fragment of at least n
45 consecutive amino acids of SEQ ID NO: 31, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, or more). These GAS 058 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 31. Preferred fragments

of (b) comprise an epitope from SEQ ID NO: 31. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 31. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of SEQ ID NO: 31 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(17) GAS 290

GAS 290 corresponds to M1 GenBank accession numbers GI:13622978 and GI:15675757, to M3 GenBank accession number GI: 21911221, to M18 GenBank accession number GI: 19746869 and is also referred to as 'Spy1959' (M1), 'SpyM3_1685' (M3), and 'SpyM18_2026' (M18). Amino acid and polynucleotide sequences of GAS 290 of an M1 strain are set forth below:

SEQ ID NO: 33

MKHILFIVGSLREGSFNHQLAAQAQKALEHQAVVSYLNWKDVVPLNQDIEANAPLPVVDARQAVQSADAI
WIFTPVYNFSIPGSVKNLDDWLSRALDLSPTGPSAIGGKVTVSSVANGGHDQVFDQFKALLPFIRTSV
AGEFTKATVNPDAWGTGRLEISKETKANLLSQAEALLAAI

SEQ ID NO: 34

ATGAAACATATTTTATTTATTGTTGGCTCGCTTCGTGAAGGGTCTTTTAACCATCAATTAGCGGCTCAAG
CACAAAAGCTCTGGAACATCAAGCAGTTGTATCTTACTTAAATTGGAAAGACGTTCTGTTTTGAATCA
AGATATCGAAGCTAATGCACCTTTACCAAGTTGTTGACGCTCGTCAAGCTGTTTCAGTCAGCGGATGCTATC
TGGATTTTACACCAGTTTACAACCTTCTCTATTCCAGGTTCTGTTAAAAACCTGCTAGACTGGTTGTCTC
GTGCTCTTGATTGTCTGATCCGACGGGCCATCTGCTATTGGCGGTAAGGTGGTTACGGTCTCTTCAGT
TGCAATGGCGGGCATGATCAAGTATTTGATCAGTTTAAAGCACTATTGCCGTTTATCCGAACCTTCAGTA
GCAGGAGAGTTTACAAAAGCAACTGTGAATCCTGATGCCTGGGGAACAGGAAGGCTTGAGATTTCAAAG
AGACAAAAGCAAACTTGCTATCTCAGGCAGAGGCTCTTTTAGCGGCTATTTAG

Preferred GAS 290 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 33; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 33, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 290 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 33. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 33. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 33. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(18) GAS 511

GAS 511 corresponds to M1 GenBank accession numbers GI:13622798 and GI:15675592, to M3 GenBank accession number GI: 21911053, to M18 GenBank accession number GI: 19746700 and is also referred to as 'Spy1743' (M1), 'SpyM3_1517' (M3), 'SpyM18_1815' (M18) and 'accA'. Amino acid and polynucleotide sequences of GAS 511 of an M1 strain are set forth below:

SEQ ID NO: 35

MTDVSRI LKEARDQGR LTTLDYANLI FDDFMELHGDRHFSDDGAIVGGLAYLAGQPVTVIGIQKGKNLQD
NLARNFGQPNPEGYRKALRLMKQAEKFGRPVVTFINTAGAYPGVGAEERGQGEAIAKNLMEMSDLKVPII
AIIIGEGGSGGALALAVADQVWMLENTMYAVLSPEGFASILWKDGSRATEAAELMKITAGELYKMGIVDR
5 I IPEHGYFSSEIVDI I KANLIEQITSLQAKPLDQLLDERYQRFKY

SEQ ID NO: 36

ATGACAGATGTATCAAGAATTTTAAAAGAAGCGCGTGATCAAGGGCGTTTAAACAACCTTGGATTACGCCA
ACCTTATTTTCGATGACTTTATGGAACGTCATGGCGATCGCCATTTTTCAGATGATGGTGCCATTGTAGG
10 TGGCCTAGCTTATTTGGCGGGACAACCTGTTACGGTCATTGGTATTCAAAAAGGTAAGAATTTACAGGAT
AATTTGGCAAGGAATTTTGGCCAGCCCAATCCAGAAGGTTATCGTAAAGCTTTGCGCCTTATGAAACAGG
CAGAAAAATTTGGACGACCAGTTGTTACGTTTATCAATACTGCAGGAGCCTATCCAGGTGTCGGTGCGGA
AGAACGAGGACAGGGTGAGGCCATTGCTAAAAATTTGATGGAAATGAGTGATCTCAAGGTTCCCATTTATC
GCCATCATTATTGGTGAAGGAGGCTCTGGTGGTGCATTAGCCTTAGCGGTTGCCGATCAGGTCTGGATGC
15 TTGAAAATACTATGTATGCGGTTCTTAGCCCAAGGCTTTGCTTCTATTTTATGGAAGGATGGTTCAAG
GGCGACCGAGGCCGCTGAATTGATGAAAATCACAGCGGGTGAACCTACAAAATGGGAATAGTAGACCGT
ATTATTCAGAACATGGTTATTTTCAAGTGAAATCGTTGACATCATCAAAGCTAACCTCATCGAACAAA
TAACCAGTTTGCAAGCTAAGCCATTAGACCAATTATTAGATGAGCGCTACCAACGCTTTCGTAAATATTA
A
20

Preferred GAS 511 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 35; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 35, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
25 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These GAS 511 proteins include variants (e.g. allelic
variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 35. Preferred fragments of (b)
comprise an epitope from SEQ ID NO: 35. Other preferred fragments lack one or more amino acids
(e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 35.
30 Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a
cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(19) GAS 533

GAS 533 corresponds to M1 GenBank accession numbers GI:13622912 and GI:15675696, to M3
GenBank accession number GI: 21911157, to M18 GenBank accession number GI: 19746804 and is
35 also referred to as 'Spy1877' (M1), 'SpyM3_1621' (M3), 'SpyM18_1942' (M18) and 'glnA'. GAS
533 has also been identified as a putative glutamine synthetase. Amino acid and polynucleotide
sequences of GAS 533 of an M1 strain are set forth below:

SEQ ID NO: 37

MAITVADIRREVKEKNVTFRLRMFTDIMGVMKNVEIPATKEQLDKVLSNKVMFDGSSIEGFVRINESDMY
40 LYPDLDTWIVFPWGDENGAVAGLICDIYTAEGKPFAGDPRGNLKRALKHMNEIGYKSFNLGPEPEPFPLFK
MDDKGNPTLEVNDNGGYFDLAPIDLADNTRREIVNILT KMGFEVEASHHEVAVGQHEIDFKYADV LKACD
NIQIFKL VVKTIAREHGLYATFMAKPKFGIAGSGMHCNMSLFDNQGNNAFYDEADKRG MQLSEDAYYFLG
GLMKHAYNYTAITNPTVNSYKRLVPGYEAPVYVAWAGSNRSPLIRVPASRGMGTRLELRSDPTANPYLA
LAVLLEAGLDGIINKIEAPEPVEANIYMTMEERNEAGIIDLPSTLHNALKALQKDDVVQKALGYHIYTN
45 FLEAKRIEWSSYATFVSQWEIDHYIHN

SEQ ID NO: 38

ATGGCAATAACAGTAGCTGACATTCGTCTGAAGTCAAAGAAAAAATGTAACGTTTCTTCGCTTGATGT
TCACTGATATCATGGGCGTTATGAAAAATGTGGAGATTCTTGCAACTAAAGAACAGTTAGACAAAGTATT
50 GTCTAACAAAGTTATGTTTGTATGGTTCATCTATCGAAGGTTTGTACGGATCAATGAGTCAGATATGTAC

CTTTACCCCGATTTAGACACTTGGATTGTTTTTCCCTGGGGAGATGAAAATGGAGCAGTTGCAGGTTTAA
TTTGTGATATTTATACAGCAGAAGGAAAGCCTTTTGCAGGAGATCCTAGAGGAAATTTAAAAAGAGCCCT
GAAACACATGAACGAGATCGGCTACAAATCATTTAATCTTGGACCAGAACCAGAATTTTTCCTTTTAAAG
ATGGATGATAAAGGTAATCCGACACTTGAAGTTAACGATAATGGTGGTTATTTTGATTAGCGCCAATTG
5 ACTTAGCAGACAACACGCGCCGTGAAATTGTGAATATTTTAACGAAAATGGGTTTTGAAGTGGAAGCTAG
TCATCATGAAGTGGCTGTTGGTCAACATGAGATTGATTTTAAATATGCAGATGTTTTGAAAGCTTGTGAT
AATATTCAAATTTTAAAGCTAGTTGTAAAAACGATTGCCCCGTGAACATGGACTTTATGCTACTTTCATGG
CTAAACCAAATTTGGAATAGCTGGATCAGGGATGCACTGTAACATGTCTTTGTTTGATAACCAAGGTAA
TAATGCTTTTATGATGAAGCTGATAAGCGAGGGATGCAGTTATCAGAAGATGCTTATTATTCTTGGGA
10 GGACTAATGAAGCATGCTTATACTACACTGCTATCACTAACCCCTACAGTGAATTCTTATAAACGATTAG
TTCCAGGTTATGAGGCACCTGTTTATGTGCTTGGGCTGGAAGTAATCGTTCACCGCTTATCCGTGTTCC
AGCATCACGTGGTATGGGAACGCGTTTGGAGTTACGTTGCGTTGATCCGACAGCTAATCCTTATTTAGCC
TTGGCTGTTCTCTTGAAGCTGGATTAGATGGTATCATTAACAAAATTGAAGCTCCAGAACCCGTTGAAG
CTAACATTTATACCATGACAATGGAAGAACGAAATGAAGCAGGCATTATTGATTTGCCATCAACGCTTCA
15 TAATGCCTTAAAAGCTCTTCAAAAAGATGATGTGGTACAAAAGGCACTAGGTTACCATATCTACACTAAT
TTCTTAGAAGCAAACGAATTGAATGGTCTTCCTATGCAACTTTTGTCTCAATGGGAATTGACCATT
ATATTCATAATTATTAG

Preferred GAS 533 proteins for use with the invention comprise an amino acid sequence: (a) having
20 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 37; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 37, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 533 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 37. Preferred fragments
25 of (b) comprise an epitope from SEQ ID NO: 37. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
NO: 37. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide,
of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

30 (20) GAS 527

GAS 527 corresponds to M1 GenBank accession numbers GI:13622332, GI:15675169, and
GI:24211764, to M3 GenBank accession number GI: 21910381, to M18 GenBank accession number
GI: 19746136, and is also referred to as 'Spy1204' (M1), 'SpyM3_0845' (M3), 'SpyM18_1155'
(M18) and 'guaA'. GAS 527 has also been identified as a putative GMP synthetase (glutamate
35 hydrolyzing) (glutamate amidotransferase). Amino acid and polynucleotide sequences of GAS 527 of
an M1 strain are set forth below:

SEQ ID NO: 39

MTEISILNDVQKIIVLDYGSQYNQLIARRIREFGVFSELKSHKITAQELREINPIGIVLSGGPNSVYADN
AFGIDPEIFELGIPILGICYGMQLITHKLGGKVVPAGQAGNREYQSTLHLRETSKLFSGTPQEQVLMS
40 HGDAVTEIPEGFHLVGDSNDCPYAAIENTEKNLYGIQFHPEVRHSVYGNLILKNFAISICGARGDWSMDN
FIDMEIAKIRETVGDRKVLGLSGGVDSSVVGVLQKAIGDQLTCIFVDHGLLRKDEGDQVMGMLGGKFG
LNIIRVDASKRFLDLLADVEDPEKKRKIIGNEFVYVFDDEASKLKGVDFLAQGTLYTDIIESGTETAQTI
KSHHNVGGLPEDMQFELIPLNTLKFDEVRLGIALGMPEEIVWRQFPFPGPLAIRVMGAITEKLETVR
ESDAILREBIAKAGLDRDVWQYFTVNTGVRSVGVMDGRTYDYTIAIRAITSIDGMTADFAQLPWDVLKK
45 ISTRIVNEVDHVNRIYDITSKPPATVEWE

SEQ ID NO: 40

ATGACTGAAATTTCAATTTTGAATGATGTTCAAAAAATTATCGTTCTTGATTATGGTAGCCAGTACAATC
AGCTTATTGCTAGACGTATTCGAGAGTTTGGTGTCTTCTCCGAACATAAAAAGCCATAAAATCACCGCTCA

AGAACTTCGTGAGATCAATCCCATAGGTATCGTTTTATCAGGAGGGCCTAACTCTGTTTACGCTGATAAC
GCCTTTGGCATTGACCCTGAAATCTTTGAACTAGGGATTCCGATTCTTGGTATCTGTTACGGTATGCAAT
TAATCACCCATAAAATTAGGTGGTAAAGTTGTTCTTGCTGGACAAGCTGGTAATCGTGAATACGGTCAGTC
AACCCTTCATCTTCGTGAAACGTCAAAATTATTTTCAGGCACACCTCAAGAACAACCTCGTTTTGATGAGC
5 CATGGTGATGCTGTTACTGAAATTCAGAAGGTTTCCACCTTGTGGAGACTCAAATGACTGTCCTATG
CAGCTATTGAAAATACTGAGAAAAACCTTTACGGTATTCAGTTCCACCCAGAAGTGAGACACTCTGTTTA
TGAAATGACATTCTTAAAACTTTGCTATATCAATTTGTGGCGCGCGTGGTGATTGGTCAATGGATAAT
TTTATTGACATGGAAATTGCTAAAATTCGTGAAACTGTAGGCGATCGTAAAGTTCTTCTAGGTCTTTCTG
GTGGAGTTGATTCTTCAGTTGTTGGTGTCTACTTCAAAAAGCTATCGGTGACCAATTAACCTGTATTTT
10 CGTTGATCACGGTCTTCTTCGTAAAGACGAGGGCGATCAAGTTATGGGAATGCTTGGGGGCAAATTTGGC
CTAAATATTATCCGTGTGGATGCTTCAAAACGTTTCTTAGACCTTCTTGCAGACGTTGAAGATCCTGAGA
AAAAACGTAAAATTATTGGTAATGAATTTGTCTATGTTTTGATGATGAAGCCAGCAAATTTAAAGGTGT
TGACTTCCTTGCCCAAGGAACACTTTATACTGATATCATTGAGTCAGGAACAGAACTGCTCAAACCATC
AAATCACATCACAATGTGGGTGGTCTCCCCGAAGACATGCAGTTTGAATTGATTGAGCCCTTAAACACTC
15 TTTTCAAAGATGAAGTTGAGCGCTTGGAAATCGCTCTTGGAAATGCCTGAAGAAATTGTTTGGCGCCAACC
ATTTCCAGGTCTCGACTTGCTATCCGTGTCATGGGAGCAATTACTGAAGAAAACTTGAACCGTTTCGC
GAATCAGACGCTATCCTTCGTGAAGAAATTGCTAAGGCTGGACTTGATCGTGACGTGTGGCAATACTTTA
CAGTTAACACAGGTGTCCGTTCTGTAGGCGTCATGGGAGATGGTCGTACTTATGATTATACCATCGCCAT
TCGTGCTATTACGTCTATTGATGGTATGACAGCTGACTTTGCTCAACTTCCTTGGGATGTCTTGAAAAA
20 ATCTCAACACGTATCGTAAATGAAGTTGACCACGTAAACCGTATCGTCTACGACATCACAAGTAAACCAC
CCGCAACAGTTGAATGGGAATAA

Preferred GAS 527 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
25 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 39; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 39, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 527 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 39. Preferred fragments
of (b) comprise an epitope from SEQ ID NO: 39. Other preferred fragments lack one or more amino
30 acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
NO: 39. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide,
of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(21) GAS 294

35 GAS 294 corresponds to M1 GenBank accession numbers GI:13622306, GI:15675145, and
GI:26006773, to M3 GenBank accession number GI: 21910357, to M18 GenBank accession number
GI: 19746111 and is also referred to as 'Spy1173' (M1), 'SpyM3_0821' (M3), 'SpyM18_1125'
(M18) and 'gid'. GAS 294 has also been identified as a putative glucose-inhibited division protein.
Amino acid and polynucleotide sequences of GAS 294 of an M1 strain are set forth below:

40 **SEQ ID NO: 41**
MSQSTATYINVIGAGLAGSEAAYQIAKRGI PVKLYEMRGVKATPQHKTTFNFAELVCSNSFRGDSLTVNAV
LLKEEMRRRLDSIIMRNGEANRVPAGGAMAVDREGYAESVTAELENHPLIEVIRGEITEIPDDAITVIATG
PLTSDALAEKIHALLNGGDGFYFYDAAAPIIDKSTIDMSKVYLKSRDKGEAAAYLNCMPMTKEEFMAFHEAL
TTAEELAPLNAFEKEKYFEGCMPIEVMAKRGIKTMLYGPMKPVGLEYPDDYTGPRDGEFKTPYAVVQLRQD
45 NAAGSLYNI VGFQTHLKWGEQKRVFQMI PGLENAEFVRYGVMHRNSYMDSPNLLTETFQSRSPNLPFFAG
QMTGVEGYVESAAAGLVAGINAARLFKREEALIFPQTTAIGSLPHYVTHADSKHFQPMNVNFGI I KELEG
PRI RDKKERYEAIASRALADLDTCLASL

SEQ ID NO: 42
50 TTGTCTCAATCAACTGCAACTTATATTAATGTTATTGGAGCTGGGCTAGCTGTTCTGAAGCTGCCTATC

AGATTGCTAAGCGCGGTATCCCCGTTAAATTGTATGAAATGCGTGGTGTCAAAGCAACACCGCAACATAA
AACCACTAATTTTGCCGAATTGGTCTGTTCCAACTCATTTCGTGGTGATAGCTTAACCAATGCAGTCGGT
CTTCTCAAAGAAGAAATGCGGCGATTAGACTCCATTATTATGCGTAATGGTGAAGCTAACCGCGTACCTG
CTGGGGGAGCAATGGCTGTTGACCGTGAGGGGTATGCAGAGAGTGTCACTGCAGAGTTGGAAAATCATCC
5 TCTCATTGAGGTCATTTCGTGGTGAAATTACAGAAATCCCTGACGATGCTATCACGGTTATCGCGACGGGA
CCGCTGACTTCGGATGCCCTGGCAGAAAAAATTCACGCGCTAAATGGTGGCGACGGATTCTATTTTACG
ATGCAGCAGCGCCTATCATTGATAAATCTACCATTGATATGAGCAAGGTTTACCTTAAATCTCGCTACGA
TAAAGGCGAAGCTGCTTACCTCAACTGCCCTATGACCAAAGAAGAATTCATGGCTTTCCATGAAGCTCTG
ACAACCGCAGAAGAAGCCCCGCTGAATGCCCTTGAAAAAGAAAAGTATTTGAAGGCTGTATGCCGATTG
10 AAGTTATGGCTAAACGTGGCATTAAAACCATGCTTTATGGACCTATGAAACCCGTTGGATTGGAATATCC
AGATGACTATACAGGTCCTCGCGATGGAGAATTTAAACGCCATATGCCGTCGTGCAATTGCGTCAAGAT
AATGCAGCTGGAAGCCTTTATAATATCGTTGGTTTCCAAACCATCTCAAATGGGGTGAGCAAAAACGCG
TTTTCCAAATGATTCCAGGGCTTGAAAATGCTGAGTTTGTCCGCTACGGCGTCATGCATCGCAATTCCTA
TATGGATTACCAAATCTTTTAACCGAAACCTTCCAATCTCGGAGCAATCCAAACCTTTTCTTTGCAGGT
15 CAGATGACTGGAGTTGAAGGTTATGTGAATCAGCTGCTTCAGGTTTAGTAGCAGGAATCAATGCTGCTC
GTTTGTTCAAAAGAGAAGAAGCACTTATTTTTCCTCAGACAACAGCTATTGGGAGTTTGCCTCATTATGT
GACTCATGCCGACAGTAAGCATTTCACCAATGAACGTCAACTTTGGCATCATCAAAGAGTTAGAAGGC
CCACGCATTTCGTGACAAAAAAGACGTTATGAAGCTATTGCTAGTCGTGCTTTGGCAGATTTAGACACCT
GCTTAGCGTCGCTTTAA

Preferred GAS 294 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 41; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 41, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
25 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 294 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 41. Preferred fragments
of (b) comprise an epitope from SEQ ID NO: 41. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
30 NO: 41. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide,
of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(22) GAS 253

GAS 253 corresponds to M1 GenBank accession numbers GI:13622611, GI:15675423, and
GI:21362716, to M3 GenBank accession number GI: 21910711, to M18 GenBank accession number
35 GI: 19746473 and is also referred to as 'Spy1524' (M1), 'SpyM3_1175' (M3), 'SpyM18_1541'
(M18) and 'murG'. GAS 253 has also been identified as a putative undecaprenyl-PP-MurNAc-
pentapeptide-UDPGlcNAc GlcNAc transferase. Amino acid and polynucleotide sequences of GAS
253 of an M1 strain are set forth below:

SEQ ID NO: 43

40 MPKKILFTGGTVGHVTLNLILIPKFIKDGWEVHYIGDKNGIEHTBIEKSLDVTFHAIAITGKLRRYFSW
QNLADVFKVALGLLQSLFIVAKLRPQALFSKGGFVSVPVVAAKLLGKPVFIHESDRSMGLANKIAYKFA
TTMYTTFEQEDQLSKVKHLGAVTKVFKDANQMPESTQLEAVKEYFSRDLKTLFFIGGSAGAHVFNQFISD
HPELKQRYNIINITGDPHLNELSSHLRYVDYVTDLYQPLMAMADLVVTRGGSNTLFELLAMAKLHLIVPL
45 GKEASRGDQLENATYFEKRGYAKQLQEPDLTLHNFQAMADLFEHQADYEATMLATKEIQSPDFFYDLLR
ADISSAIKEK

SEQ ID NO: 44

ATGCCTAAGAAGATTTTATTTACAGGTGGTGGAACTGTAGGTCATGTCACCTTGAACCTCATTCTCATAC
CAAATTTATCAAGGACGGTTGGGAAGTACATTATATTGGTGATAAAAATGGCATTGAACATACAGAAAT

TGAAAAGTCAGGCCTTGACGTGACCTTTCATGCTATCGCGACAGGCAAGCTTAGACGCTATTTTTCATGG
CAAAATCTAGCTGATGTTTTTAAGGTTGCACTTGGCCTCCTACAGTCTCTCTTTATTGTTGCCAAGCTTC
GCCCTCAAGCCCTTTTTTCCAAAGGTGGTTTTGTCTCAGTACCGCCAGTTGTGGCTGCTAAATTGCTTGG
TAAACCAGTCTTTATTCATGAATCAGATCGGTCAATGGGACTAGCAAACAAGATTGCCTACAAATTTGCA
5 ACTACCATGTATACCACTTTTGAGCAGGAAGACCAGTTGTCTAAAGTTAAACACCTTGGAGCGGTGACAA
AGGTTTTCAAAGATGCCAACCAATGCCTGAATCAACTCAGTTAGAGGCGGTGAAAGAGTATTTTAGTAG
AGACCTAAAAACCCTCTTGTTTATTGGTGGTTCGGCAGGGGGCGCATGTGTTTAATCAGTTTATTAGTGAT
CATCCAGAATTGAAGCAACGTTATAATATCATCAATATTACAGGAGACCCTCACCTTAATGAATTGAGTT
CTCATCTGTATCGAGTAGATTATGTTACCGATCTCTACCAACCTTTGATGGCGATGGCTGACCTTGAGT
10 GACAAGAGGGGGCTCTAATACACTTTTTGAGCTACTGGCAATGGCTAAGCTACACCTCATCGTTCCTCTT
GGTAAAGAAGCTAGCCGTGGCGATCAGTTAGAAAATGCCACTTATTTTGAGAAGAGGGGGCTACGCTAAAC
AATTACAGGAACCTGATTTAACTTTGCATAATTTTGATCAGGCAATGGCTGATTTGTTTGAACATCAGGC
TGATTATGAGGCTACTATGTTGGCAACTAAGGAGATTCAGTCACCGGACTTCTTTTATGACCTTTTGAGA
GCTGATATTAGCTCCGCGATTAAGGAGAAGTAA

15 Preferred GAS 253 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 43; and/or (b) which is a fragment of at least *n*
consecutive amino acids of SEQ ID NO: 43, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
20 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 253 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 43. Preferred fragments
of (b) comprise an epitope from SEQ ID NO: 43. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
25 NO: 43. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide,
of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(23) GAS 529

GAS 529 corresponds to M1 GenBank accession numbers GI:13622403, GI:15675233, and
GI:21759132, to M3 GenBank accession number GI: 21910446, to M18 GenBank accession number
30 GI: 19746203 and is also referred to as 'Spy1280' (M1), 'SpyM3_0910' (M3), 'SpyM18_1228'
(M18) and 'glmS'. GAS 529 has also been identified as a putative L-glutamine-D-fructose-6-
phosphate aminotransferase (Glucosamine-6-phosphate synthase). Amino acid and polynucleotide
sequences of GAS 529 of an M1 strain are set forth below:

SEQ ID NO: 45

35 MCGIVGVVGNRNATDILMQGLEKLEYRGYDSAGIFVANANQTNLIKSVGRIADLRKIGIDVAGSTGIGH
TRWATHGQSTEDNAHPHTSQTGRFVLVHNGVIENYLHIKTEFLAGHDFKGQTDTEIAVHLIGKFVBEEDKL
SVLEAFKKSLSIIEGSYAFALMDSQATDTIYVAKNKSPLLI GLGEGYNMVCSDAMAMIRETSEFMBIHDK
ELVILTKDKVTVDYDGKELIRDSYTAELDLSDIGKGYTPFYMLKEIDEQPTVMRQLISTYADETGNVQV
DPAIITSIQEADRLYLAAAGTSYHAGFATKNMLEQLTDPVELGVASEWGYHMPLLSKKPMFILLSQSGE
40 TADSRQVLVKANAMGIPSLTVTNVPGSTLSREATYTMLIHAGPEIAVASTKAYTAQIAALAFKAVGEA
NGKQEALDFNLVHLSLVAQSI EATLSEKDLVABKVQALLATTRNAFYI GRGNDYYVAMEAALKLKEISY
IQCEGFAAGELKHGTISLIEEDTPVIALISSQLVASHTRGNIQEVAAARGAHVLT VVEGLDREGDDIIV
NKVHPFLAPIAMVIPTQLIAYYASLQRLDVKPRNLAKAVTVE

SEQ ID NO: 46

45 ATGTGTGGAATTGTTGGAGTTGTTGGAAATCGCAATGCAACGGATATTTTAATGCAAGGCCTTGAAAAGC
TTGAATACCGGGTTATGATTGAGCAGGAATTTTGTGGCTAATGCCAATCAAACAACTTGATTAAATC
AGTGGGGCGGATTGCTGATTTGCGTGCCAAGATTGGCATTGATGTTGCTGGTTCAACAGGGATTGGTCAC
ACCCGTTGGGCAACGCATGGCCAATCAACAGAGGATAATGCCCATCCTCACACGTCACAAACTGGACGTT

TTGTACTTGTTCATAATGGTGTGATTGAAAATTACCTTCACATTAAACAGAGTTCCTAGCTGGACATGA
TTTTAAGGGGCAGACAGATACTGAGATTGCAGTACACTTGATTGGAAAATTGTGGAAGAAGACAAGTTG
TCAGTACTGGAAGCTTTTAAAAAATCTTTAAGCATTATTGAAGGTTCTACGCCTTTGCATTAATGGATA
GCCAAGCAACTGATACTATTTATGTGGCTAAAAACAAGTCTCCATTGTTGATTGGACTTGGTGAAGGTTA
5 CAACATGGTTTTGTTCAGATGCCATGGCCATGATTCGTGAAACCAGTGAATTTATGGAAATTCATGATAAG
GAGCTAGTTATTTTAACCAAAGATAAGGTAAGTGTACAGACTACGATGGTAAAGAGCTGATACGAGATT
CCTACACTGCTGAATTAGACTTATCTGATATTGGCAAAGGGACTTATCCTTTCTATATGCTGAAAGAAAT
TGATGAGCAACCAACCGTAATGCGTCAATTAATTTCAACTTATGCAGATGAAACTGGTAACGTACAGGTT
GATCCGGCTATCATTACCTCTATCCAAGAGGCTGACCGTCTTTATATTTTAGCGGCAGGGACTTCCTACC
10 ATGCTGGTTTTGAACAAAAAATATGCTTGAGCAATTGACAGATACACCAGTTGAGTTGGGCGTGGCTTC
TGAGTGGGGTTACCACATGCCTCTGCTTAGCAAGAAACCAATGTTTATTCTACTAAGCCAATCAGGAGAA
ACCGCAGATAGTCGTCAAGTTTATAGTAAAGGCAAATGCTATGGGCATTCCGAGTTTGACAGTAACTAACG
TTCCAGGATCAACCTTATCACGTGAAGCAACATACACCATGTTGATTGATGCTGGACCTGAAATTGCTGT
TGCGTCTACAAAAGCTTACACTGCACAAATTGCTGCCCTTGCCTTTTTGGCTAAGGCAGTTGGTGAGGCA
15 AATGGTAAGCAAGAAGCTCTTGACTTTAACTTGGTACATGAGTTGTCATTGGTTGCCCAATCTATTGAGG
CGACTTTGTCTGAAAAAGATCTCGTGGCAGAAAAGGTTCAAGCTTTGCTAGCTACTACTCGTAATGCTTT
TTACATCGGGCGTGGCAATGATTATTACGTTGCGATGGAAGCTGCTTTGAAATTAAAAGAGATTTCTTAT
ATTCAATGCGAAGGCTTTGCGGCTGGTGAATTGAAACATGGAACCATTTCAATTAATTGAGGAGGACACGC
CAGTAATCGCTTTAATATCGTCTAGTCAGTTGGTTGCCTCTCATACGCGTGGTAATATTCAAGAAGTTGC
20 TGCCCGTGGGGCTCATGTTTTAACAGTTGTGGAAGAAGGGCTTGACCGTGAGGGAGATGACATTATTGTC
AATAAGGTTTCATCCTTTCTAGCCCCGATTGCTATGGTCATTCCAACTCAACTGATTGCTTACTACGCTT
CATTACAACGTGGACTTGATGTTGATAAGCCACGTAATTTGGCTAAAGCTGTAACAGTAGAATAA

Preferred GAS 529 proteins for use with the invention comprise an amino acid sequence: (a) having
25 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 45; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 45, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 529 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralog, mutants, etc.) of SEQ ID NO: 45. Preferred fragments
30 of (b) comprise an epitope from SEQ ID NO: 45. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
NO: 45. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide,
of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

35 (24) GAS 045

GAS 117 corresponds to M3 GenBank accession number GI: 21909751, M18 GenBank accession
number GI: 19745421 and is referred to as 'SpyM3_0215' (M3), 'SpyM18_oppA' (M18) and 'oppA'.
GAS 045 has been identified as an oligopeptide permease. Amino acid and polynucleotide sequences
of GAS 045 from an M1 strain are set forth below:

40 SEQ ID NO: 47

VTFMKKSKWLA AVSVAILSVSALAACGNKNASGGSEATKTYKYVFNDPKSLDYILTNGG
GTTDVI TQMV DGLLENDEYGNLVPSLAKDWKVS KDGLTYTYTLRDGVSWYTADGEBYAPV
TAEDFVTGLKHAVDDKSDALYVVEDSIKNL KAYQNGEVDPFKEVGVKALDDKT VQYTLNKP
ESYWN SKTTYSVLFPVNAKFLKSKGKDFGTTDPSSILVNGAYFLSAFTSKSSMEFHKNEN
45 YWDAKNVGI ESVKLTYS DGS DPGSFYKNFDKGEFSVARLYPNDPTYKSAKKNYADNITYG
MLTGDI RHLTWN LNRTSFKNTKKDPAQQDAGKKALNNKDFRQAIQFAFDRASFQAQTAGQ
DAKTALRNMLVPPTFVTIGESDFGSEVEKEMAKLGDEWKDVNLADAQDGFYNPEKAKAE
FAKAKEALTAEGVTFPVQLDYPVDQANAATVQEAQSFKQSVEASLGKENVI VNVLETETS
THEAQGFYAETPEQQDYDI I SSWWGP DYQDPRTYLDIMSPVGGGSVIQKLGIKAGQNKDV
50 VAAAGLD TYQTL LDEAAAI TDDNDARYKAYAKAQAYLT DNAV DI PVVALGGT PRVTKAVP
FSGGFSWAGSKGPLAYKGMKLQDKPVTVKQYBKAKEKWMKAKAKSNAKYAEKLADHVEK

SEQ ID NO: 48

GTGACTTTTATGAAGAAAAGTAAATGGTTGGCAGCTGTAAGTGTTCGATCTTGTCAGTA
TCCGCTTTGGCAGCTTGTGGTAATAAAAATGCTTCAGGTGGCTCAGAAGCTACAAAACC
5 TACAAGTACGTTTTTGTAAACGATCCAAAATCATTGGATTATATTTGACTAATGGCGGT
GGAACGACTGATGTGATAACACAAATGGTTGATGGTCTTTTGAAAACGATGAGTATGGT
AATTTAGTACCATCACTTGCTAAAGATTGGAAGGTTTCAAAAGACGGTCTGACTTATACT
TATACTCTTCGCGATGGTGTCTCTTGGTATACGGCTGATGGTGAAGAATATGCCCCAGTA
ACAGCAGAAGATTTTGTGACTGGTTTGAAGCACGCGTTGACGATAAATCAGATGCTCTT
10 TACGTTGTTGAAGATTCAATAAAAACTTAAAGGCTTACCAAAATGGTGAAGTAGATTTT
AAAGAAGTTGGTGTCAAAGCCCTTGACGATAAACTGTTTCACTATCTTTGAACAAGCCT
GAAAGCTACTGGAATTCAAAACACTTATAGTGTGCTTTTCCAGTTAATGCGAAATTT
TTGAAGTCAAAAGGTAAAGATTTTGGTACAACCGATCCATCATCAATCCTTGTTAATGGT
GCTTACTTCTTGAGCGCCTTCACCTCAAAATCATCTATGGAATTCATAAAAATGAAAAC
15 TACTGGGATGCTAAGAATGTTGGGATAGAATCTGTAAATTGACTTACTCAGATGGTTCA
GACCCAGGTTTCGTTCTACAAGAATTTGACAAGGGTGAGTTCAGCGTTGCACGACTTTAC
CCAAATGACCCTACCTACAAATCAGCTAAGAAAACTATGCTGATAACATTACTTACGGA
ATGTTGACTGGAGATATCCGTCATTTAATCATGGAATTTGAACCGTACTTCTTTCAAAAAC
ACTAAGAAAGACCCTGCACAACAAGATGCCGGTAAGAAAGCTCTTAACAACAAGGATTTT
20 CGTCAAGCTATTCAGTTTGCTTTTGACCGAGCGTCATTCCAAGCACAACTGCAGGTCAA
GATGCCAAAACAAAAGCCTTACGTAACATGCTTGTCACCAACATTTGTGACCATTTGGA
GAAAGTGATTTTGGTTGAGAAGTTGAAAAGGAAATGGCAAACTTGGTGATGAATGGAAA
GACGTTAACTTAGCTGATGCTCAAGATGGTTTCTATAATCCTGAAAAGCAAAAGCTGAG
TTTGCAAAAGCCAAAGAAGCTTTAACAGCTGAAGGTGTAACCTTCCAGTTCAATTAGAT
25 TACCCTGTTGACCAAGCAAACGCAGCAACTGTTTCAAGGAGCCAGTCTTTCAAACAATCT
GTTGAAGCATCTCTTGGTAAAGAGAATGTCAATTGTCAATGTTCTTGAAACAGAAACATCA
ACTCACGAAGCCCAAGGCTTCTATGCTGAGACCCCAAGCAACAAGACTACGATATCATT
TCATCATGGTGGGACCAGACTATCAAGATCCACGGACCTACCTTGACATCATGAGTCCA
GTAGGTGGTGGATCTGTTATCCAAAACCTTGAATCAAAGCAGGTCAAATAAGGATGTT
30 GTGGCAGCTGCAGGCCTTGATACCTACCAAACCTTCTTGTGATGAAGCAGCAGCAATTACA
GACGACAACGATGCGCGCTATAAAGCTTACGCAAAAGCACAAGCCTACCTTACAGATAAT
GCCGTAGATATTCCAGTTGTGGCATTGGGTGGCACTCCACGAGTTACTAAAGCCGTTCCA
TTTAGCGGGGGCTTCTCTTGGGCAGGGTCTAAAGGTCTCTAGCATATAAAGGAATGAAA
CTTCAAGACAAACCTGTCAAGTAAACAATACGAAAAGCAAAAGAAAATGGATGAAA
35 GCAAAGGCTAAGTCAAATGCAAATATGCTGAGAAGTTAGCTGATCACGTTGAAAAA

Preferred GAS 045 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 47; and/or (b) which is a fragment of at least *n*
40 consecutive amino acids of SEQ ID NO: 47, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 045 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 47. Preferred fragments
of (b) comprise an epitope from SEQ ID NO: 47. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
45 amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
NO: 47. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of
SEQ ID NO: 47 is removed. Other fragments omit one or more domains of the protein (e.g. omission
of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular
domain).

50 (25) GAS 095

GAS 095 corresponds to M1 GenBank accession numbers GI:13622787 and GI:15675582, to M3
GenBank accession number GI: 21911042, to M18 GenBank accession number GI: 19746634 and is

also referred to as 'Spy1733' (M1), 'SpyM3_1506' (M3), 'SpyM18_1741' (M18). GAS 095 has also been identified as a putative transcription regulator. Amino acid and polynucleotide sequences of GAS 095 of an M1 strain are set forth below:

SEQ ID NO: 49

5 MKIGKKIVLMFTAIVLTTVLALGVYLTSAYTFSTGELSKTFKDFSTSSNKSDAIKQTRAFSILLMGVDTG
SSERASKWEGNSDSMILVTVPKTKKTTMTSLERDTLTTLSGPKNNEMNGVEAKLNAAAYAAGGAQMAIMT
VQDLLNITIDNYVQINMQGLIDLNAVGGITVTNEFDPIISIAENEPEYQATVAPGTHKINGEQALVYAR
MRYDDPEGDYGRQKRQREVIQKVLKKILALDSISSYRKILSAVSSNMQTNIEISSRTIPSLGYPDALRT
10 IKTYQLKGEDATLSGGSYQIVTSNHLLEIQNRIRTELGLHKVNQLKTNATVYENLYGSTKSQTVNNNYD
SSGQAPSYSDSHSSYANYSSGVDGTQASASTDQDSTASSHRPATPSSSSDALAADESSSSSGSGSLVPPANI
NPQT

SEQ ID NO: 50

15 ATGAAAATTGGAAAAAATAGTTTTAATGTTACAGCTATTGTGTTAACTGTCTTGGCATTAGGTG
TCTATCTAACTAGTGCTTATACCTTCTCAACAGGAGAATTATCAAAGACCTTTAAAGATTTTTCGACATC
TTCAAACAAAAGTGATGCCATTAAACAAACAAGAGCTTTTCTATCTTGTGATGGGTGTTGATACAGGC
TCTTCAGAGCGTGCCTCCAAGTGGGAAGGAAACAGTGATTTCGATGATTTTGGTTACGGTTAATCCAAAGA
CCAAGAAAACAACTATGACTAGTTTAGAACGAGATACCTTAACCACGTTATCTGGACCCAAAAATAATGA
AATGAATGGTGTGTAAGCTAAGCTTAACGCTGCTTATGCAGCAGGTGGCGCTCAGATGGCTATTATGACC
20 GTGCAAGATCTTTTGAATATCACCATTGATAACTATGTTCAAATTAATATGCAAGGCCTTATTGATCTTG
TGAATGCAGTTGGAGGGATTACAGTTACAAATGAGTTTGATTTTCTATCTCGATTGCTGAAAACGAACC
TGAATATCAAGCTACTGTTGCGCCTGGAACACACAAAATTAACGGTGAACAAGCTTTGGTTTATGCTCGT
ATGCGTTATGATGATCCTGAGGGAGATTATGGTCGACAAAAGCGTCAACGTGAAGTCATTCAAAGGTAT
TGAAAAAATCCTTGCTCTTGATAGCATTAGCTCTTATCGGAAGATTTTATCTGCTGTAAGTAGTAATAT
25 GCAAACGAATATCGAAATCTTCTCGCACTATCCCTAGTCTATTAGGTTATCGTGACGCACCTTAGAACT
ATTAAGACTTATCAACTAAAAGGAGAAGATGCCACTTTATCAGATGGTGGATCATACCAAATTGTTACCT
CTAATCATTTGTTAGAAATCCAAATCGTATCCGAACAGAATTAGGACTTCATAAGGTTAATCAATTAAA
AACAAATGCTACTGTTTATGAAAATTTGTATGGGTCAACTAAGTCTCAGACAGTAAACAACAACATGAC
TCTTCAGGCCAGGCTCCATCTTATCTGATAGTCATAGCTCTTACGCTAATTATTCAAGTGGAGTAGATA
30 CCGGCCAGAGTGCTAGTACAGACCAGGACTCTACTGCTTCAAGCCATAGGCCAGCTACGCCGTCTTCTTC
ATCAGATGCTTTAGCAGCTGATGAGTCTAGCTCATCAGGGTCTGGATCATTAGTTCCTCTGCTAATATC
AACCTCAGACCTAA

Preferred GAS 095 proteins for use with the invention comprise an amino acid sequence: (a) having
35 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 49; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 49, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 095 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 49. Preferred fragments
40 of (b) comprise an epitope from SEQ ID NO: 49. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
NO: 49. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of
SEQ ID NO: 49 is removed. Other fragments omit one or more domains of the protein (e.g. omission
45 of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular
domain).

(26) GAS 193

GAS 193 corresponds to M1 GenBank accession numbers GI:13623029 and GI:15675802, to M3
GenBank accession number GI: 21911267, to M18 GenBank accession number GI: 19746914 and is

also referred to as 'Spy2025' (M1), 'SpyM3_1731' (M3), 'SpyM18_2082' (M18) and 'isp'. GAS 193 has also been identified as an immunogenic secreted protein precursor. Amino acid and polynucleotide sequences of GAS 193 of an M1 strain are set forth below:

SEQ ID NO: 51

5 MKKRKLLAVTLLSTILLNSAVPLVVADTSLRNSTSSDQPTTADTDTTDESETPKKDKKSKETASQHD
KDHKPSHTHTPTPPSNDTKQTDQASSEATDKPNKDKNDTKQPDSSDQSTPSPKDQSSQKESQNKDGRPTPS
PDQKQDQTPDKTPEKSADKTPEKGPEKATDKTPEPNRDAPKPIQPPLAAAPVFI PWRESKDLSKLPSS
RSSAAYVRHWTGDSAYTHNLLSRRYGITAEQLDGFNLGLIHYDKERLNGKRLLEWEKLTGLDVRAIVAI
10 AMAESSLGTVQVAKKGANMFGYGAFDFNPNNAKKYSDEVAIRHVMEDTIIANKNQTFERQDLKAKKWSL
GQLDTLIDGGVYFTDTSGSGQRRADIMTKLDQWIDDHGSTPBIPEHLKITSQTGFSEVPVGYKRSQPQNV
LTYKSETYSFGQCTWYAYNRVKELGYQVDRYMGNGGDWQRKPGFVTTHKPKVGYVVSFAPGQAGADATYG
HVAVVEQIKEDGSILISESNVMGLGTISYRTFTAEQASLLTYVVGDKLPRP

SEQ ID NO: 52

15 ATGAAGAAAAGGAAATTGTTAGCAGTAACACTATTAAGTACCATACTCTTAAACAGTGCAGTGCCATTAG
TTGTTGCTGATACCTCCTTGCGTAATAGCACATCATCCACTGATCAGCCTACTACAGCAGATACTGATAC
GGATGACGAGAGTGAAACACCAAAAAAGACAAAAAAGCAAGGAAACAGCGTCGCAGCACGACACCCAA
AAAGACCATAAGCCATCACACACTCACCAACCCCCCTTCAAATGATACTAAGCAGACCGATCAGGCAT
CATCTGAAGCTACTGACAAACCAAATAAAGACAAAAACGACACCAAGCAACCAGACAGCAGTGATCAATC
20 CACCCCATCTCCCAAAGACAGTCGTCTCAAAAAGAGTCACAAAACAAAGACGGCCGACCTACCCCATCA
CCTGATCAGCAAAAAGATCAGACACCTGATAAAACACCAGAAAAATCAGCTGATAAAACCCCTGAAAAAG
GACCAGAAAAAGCAACTGATAAAACACCAGAGCCAAATCGTGACGCTCCAAAACCCATCCAACCTCCTTT
AGCAGCTGCTCCTGTCTTTATACCTTGGAGAGAAAGTGACAAAGACCTGAGCAAGCTAAAACCAAGCAGT
CGCTCATCAGCGGCTTACGTGAGACACTGGACAGGTGACTCTGCCTACACTCACAACCTGTTGTCACGCC
25 GTTATGGGATTACTGCTGAACAGCTAGATGGTTTTTTGAACAGTCTAGGTATTCATATGATAAAGAACG
CTTAAACGGAAAGCGTTTATTAGAATGGGAAAACTAACAGGACTAGACGTTTCGAGCTATCGTAGCTATT
GCAATGGCAGAAAGCTCACTAGGTACTCAGGGAGTTGCTAAAGAAAAAGGAGCCAATATGTTTGGTTATG
GCGCCTTTGACTTCAACCCAAACAATGCCAAAAAATACAGCGATGAGGTTGCTATTCGTACATGGTAGA
AGACACCATCATTTGCCAACAAAAACCAACCTTTGAAAGACAAGACCTCAAAGCAAAAAAATGGTCACTA
30 GGCCAGTTGGATACCTTGATTGATGGTGGGGTTTACTTTACAGATACAAGTGGCAGTGGGCAAGACGAG
CAGATATCATGACCAAACTAGACCAATGGATAGATGATCATGGAAGCACACCTGAGATTCCAGAACATCT
CAAGATAACTTCCGGGACACAATTTAGCGAAGTGCCCGTAGGTTATAAAGAAGTCAGCCACAAAACGTT
TTGACCTACAAGTCAGAGACCTACAGCTTTGGCCAATGCACTTGGTACGCCTATAATCGTGTCAAAGAGC
TAGGTTATCAAGTCGACAGGTACATGGGTAACGGTGGCGACTGGCAGCGCAAGCCAGGTTTTGTGACCAC
35 CCATAAACCTAAAGTGGGCTATGTCTGCTCATTTCACCAGGCCAAGCAGGAGCAGATGCAACCTATGGT
CACGTTGCTGTTGTAGAGCAAAATCAAAGAAGATGGTTCTATCTTAATTTACAGAGTCAAATGTTATGGGAC
TAGGCACCATTTCTATCGGACGTTACAGCTGAGCAGGCTAGTTTGTGACCTATGTCTAGGGGACAA
ACTCCCAAGACCATAA

40 Preferred GAS 193 proteins for use with the invention comprise an amino acid sequence: (a) having
50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 51; and/or (b) which is a fragment of at least *n*
consecutive amino acids of SEQ ID NO: 51, wherein *n* is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 193 proteins include variants (e.g.
45 allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 51. Preferred fragments
of (b) comprise an epitope from SEQ ID NO: 51. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
NO: 51. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide,
50 of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(27) GAS 137

GAS 137 corresponds to M1 GenBank accession numbers GI:13621842, GI:15674720 and GI:30173478, to M3 GenBank accession number GI:21909998, to M18 GenBank accession number GI: 19745749 and is also referred to as 'Spy0652' (M1), 'SpyM3_0462', and 'SpyM18_0713' (M18).

5 Amino acid and polynucleotide sequences of GAS 137 of an M1 strain are set forth below:

SEQ ID NO: 53

MSDKHINLVIIVTGMMSGAGKTVAIQSFEDLGYFTIDNMPPALVPKFLELIEQTNENRRVALVDMRSRLFF
KEINSTLDSIESNPSIDFRILFLDATDGELVSRYKETRRSHPLAADGRVLDGIRLERELLSPLKSMSQHV
VDTTKLTTPRQLRKTISDQFSEGSNQASFRIEVMFSGFKYGLPLDADLVFDVRFNPNYYQVELREKTGLD
10 EDVFNVMSPHPESEVFYKHLNLIVPIILPAYQKEGKSVLTVAIGCTGGQHRVAFHCLAESLATDWSVN
ESHRDQNRKRVNRS

SEQ ID NO: 54

ATGTCAGACAAACACATTAATTTAGTTATTGTGACAGGAATGAGCGGCGCTGGAAAAACAGTTGCCATTC
15 AGTCTTTTGAGGATCTAGGCTACTTTACCATTGATAATATGCCCCAGCCTTGGTTCCAAAATTTTGA
ATTAATTGAACAAACCAATGAAAATCGTAGGGTGGCTTTGGTTGTCGATATGAGAAGTCGTTTGTTC
AAGGAAATTAATTCTACCTTAGATAGTATTGAAAGCAATCCTAGCATTGATTTTCGGATTCTTTTTTGG
ATGCAACGGATGGAGAATTGGTGTACGCTATAAAGAAACCAGACGGAGCCACCCTTTGGCTGCGGACGG
TCGTGTGCTTGATGGTATTGATTTGGAAAGAGAACTCCTATCTCCTTTGAAAAGCATGAGCCAACATGTG
20 GTGGATACAACAAAATTGACCCCTAGACAATTGCGTAAAACCATTTTCAGACCAGTTTCTGAAGGGTCTA
ATCAAGCCTCTTTCCGTATTGAAGTGATGAGCTTTGGGTTCAAATATGGTCTTCCTTTGGATGCGGATTT
GGTTTTTGATGTGCGTTTTCTACCCAATCCTTATTATCAGGTAGAGCTTCGTGAAAAAACAGGACTAGAT
GAGGACGTTTTTAATTATGTGATGTCTCACCAGAATCAGAGGTGTTTACAAGCATTGTAAACCTTA
TTGTCCCTATCTTACCGGCTTACCAAAAAGAAGGGAAGTCTGTCTTGACGGTGGCTATTGGCTGCACAGG
25 AGGCCAACACCGCAGCGTTGCCTTTGCCCATTTGCTTGGCAGAAAGTCTGGCAACAGATTGGTCGGTTAAT
GAAAGCCATCGTGATCAAAATCGTCGTAAGGAAACGGTGAATCGTTCATGA

Preferred GAS 137 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
30 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 53; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 53, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 137 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 53. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 53. Other preferred fragments lack one or more amino
35 acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 53. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(28) GAS 084

40 GAS 084 corresponds to M1 GenBank accession numbers GI:13622398 and GI:15675229, to M3 GenBank accession number GI: 21910442, to M18 GenBank accession number GI: 19746199 and is also referred to as 'Spy1274' (M1), 'SpyM3_0906' and 'SpyM18_1223' (M18). GAS 084 has also been identified as a putative amino acid ABC transporter/periplasmic amino acid binding protein. Amino acid and polynucleotide sequences of GAS 084 of an M1 strain are set forth below:

45 **SEQ ID NO: 55**

MIKKRTVAILAIASSFFLVACQATKSLKSGDAWGVYQKQKSITVGFNDTFVPMGYKDESGRCKGFDIDL

AKEVFHQYGLKVNFAQINWDMKEAELNNGKIDVIWNGYSITKERQDKVAFTDSYMRNEQIIIVVKKRSDIK
TISDMKHVKVLGAQSASSGYDSLRLTPKLLKDFIKNKDANQYETFTQAFIDLKSDRIDGILIDKVYANYYL
AKEGQLENYRMIPTTFENEAFSVGLRKEDKTLOAKINRAFRVLYQNGKFQAISEKWFGDDVATANIKS

5 SEQ ID NO: 55

ATGATTATAAAAAAAGAACCGTAGCAATTTTAGCCATAGCTAGTAGCTTTTCTTGGTAGCTTGTCAAG
CTACTAAAAGTCTTAAATCAGGAGATGCTTGGGGAGTTTACCAAAGCAAAAAAGTATTACAGTTGGTTT
TGACAATACGTTTGTTCCTATGGGCTATAAGGATGAAAGCGGCAGATGCAAAGGTTTGTATATTGATTTG
GCTAAAGAAGTTTTTCACCAATATGGACTCAAGGTAACTTTCAAGCTATTAATTGGGACATGAAAGAAG
10 CAGAACTAAACAATGGTAAAATTGATGTAATCTGGAATGGTTATTCAATAACTAAGGAGCGTCAGGATAA
GGTTGCCTTTACTGATTCTTACATGAGAAATGAACAAATTATTGTTGTCAAAAAAGATCTGATATTAAA
ACAATATCAGATATGAAACATAAAGTGTAGGAGCACAATCAGCTTCATCAGGTTATGACTCCTTGTTAA
GAACTCCTAAACTGCTGAAAGATTTTATTAAAAATAAAGACGCTAATCAATATGAAACCTTTACACAAGC
TTTTATTGATTTAAAATCAGATCGTATCGATGGAATATTGATTGACAAAGTATATGCCAATTACTATTTA
15 GCAAAAGAAGGGCAATTAGAGAATTATCGGATGATCCCAACGACCTTTGAAAATGAAGCATTTTCGGTTG
GACTTAGAAAAGAAGACAAAACGTTGCAAGCAAAAATTAATCGTGCTTTCAGGGTGCTTTATCAAAATGG
CAAATTTCAAGCTATTTCTGAGAAATGGTTTGGAGATGATGTTGCCACTGCCAATATTAAATCTTAA

Preferred GAS 084 proteins for use with the invention comprise an amino acid sequence: (a) having
20 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 55; and/or (b) which is a fragment of at least n
consecutive amino acids of SEQ ID NO: 55, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25,
30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 084 proteins include variants (e.g.
allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 55. Preferred fragments
25 of (b) comprise an epitope from SEQ ID NO: 55. Other preferred fragments lack one or more amino
acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more
amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
NO: 55. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of
SEQ ID NO: 55 is removed. Other fragments omit one or more domains of the protein (e.g. omission of
30 a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(29) GAS 384

GAS 384 corresponds to M1 GenBank accession numbers GI:13622908 and GI:15675693, to M3
GenBank accession number GI: 21911154, to M18 GenBank accession number GI: 19746801 and is
also referred to as 'Spy1874' (M1), 'SpyM3_1618' (M3), and 'SpyM18_1939' (M18). GAS 384 has
35 also been identified as a putative glycoprotein endopeptidase. Amino acid and polynucleotide
sequences of GAS 384 of an M1 strain are set forth below:

SEQ ID NO: 57

MKTLAFDTSNKTLSLAILDDETLADMTLNIQKKHSVSLMPAIDFLMTCTDLKPQDLERIVVAKGPGSYT
GLRVAVATAKTLAYSLNIALVGISSLYALAASTCKQYPNTLVVPLIDARRQNAYVGYRQKSVMPQAHA
40 SLEVIIEQLVBEGQLIFVGETAPFABKIQKKLPQAILLPTLPAYECGLLGQSLAPENVDAFVPQYLKRV
BAEENWLKDNEIKDDSHYVKRI

SEQ ID NO: 58

ATGAAGACACTTGCAATTTGATACCTCAAATAAAACCTTGTCCTTGCTATACTTGATGATGAGACACTTC
45 TAGCAGATATGACCCTTAACATTACAGAAAAACATAGTGTTAGCCTTATGCCTGCTATTGATTTTTTGAT
GACTTGTAAGTATGCTTAAACCTCAAGATTTAGAAAGAATAGTGGTTGCAAAAGGCCCTGGATCTTACACA
GGTTTACGAGTGGCAGTTGCTACTGCAAAACGTTAGCGTACAGTTTAAATATTGCATTGGTCCGGGATTT
CGAGTCTATATGCTTTGGCTGCGTCTACTTGTAACAGTATCCAAATACTTTGGTGGTGCCATTGATTGA
TGCTAGAAGGCAAAATGCGTATGTAGGTTATTATCGGCAAGGAAATCAGTGATGCCACAAGCCCATGCT

TCACTAGAAGTTATTATAGAACAATTAGTAGAAGAAGGACAGCTGATTTTTGTTGGGGAGACTGCTCCTT
TTGCTGAGAAAATTCAAAAGAACTACCTCAGGCGATACTACTTCCAACCCTTCCTTCTGCTTACGAATG
TGGTCTTTTGGGGCAAAGTTTGGCACCAGAAAATGTAGACGCCTTTGTCCCTCAATATCTCAAGAGAGTG
GAAGCTGAAGAAAAGTGGCTCAAAGATAATGAGATAAAAGATGATAGTCACTACGTTAAGCGAATCTAA

5 Preferred GAS 384 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 57; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 57, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 384 proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 57. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 57. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID
10 NO: 57. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(30) GAS 202

GAS 202 corresponds to M1 GenBank accession numbers GI:13622431 and GI:15675258, to M3 GenBank accession number GI: 21910527, to M18 GenBank accession number GI: 19746290 and is
20 also referred to as 'Spy1309' (M1), 'SpyM3_0991' (M3), 'SpyM18_1321' (M18) and 'dltD'. GAS 202 has also been identified as a putative extramembranal protein. Amino acid and polynucleotide sequences of GAS 202 of an M1 strain are set forth below:

SEQ ID NO: 59

MLKRLWLIIGPLLI AFVLVVITIFSPTQLDHSIAQEKANAVAITDSSFKNGLIKRQALSDETCRFVPPF
25 GSSEWSRMDSMHPSVLAERYKRSYRPFLLIGKRGASLSHYGIQQITNEMQKKKAI FVVSPQWPTAQGIN
PSAVQMYLSNTQVIEFLLKARTDKESQFAAKRLLELNPVSKSNLLKKVSKGKSLRLDRAILKCQHQA
LREESLFSFLGKSTNYEKRI LPRVKGLPKVFSYKQLNALATKRGQLATTNNRFGIKNTFYRKRIAPKYNL
YKNFQVNYSYLASPEYNDFQLLLSEFAKRKTDVLFVITPVNKAWADYTGLNQDKYQAAVRKIKFQLKSQG
FHRIADFSKDGGSYFMQDTIHLGWNGWLA FDKKVQPFLETQKQVPNYKMNPYFYSKIWANRKDLQ

SEQ ID NO: 60

ATGCTTAAGAGACTCTGGTTAATTCTAGGTCCTCTTCTTATTGCCTTTGTTTTAGTAGTGATTACTATTT
TTAGTTTTCTACACAACCTTGATCATTCCATAGCTCAGGAAAAAGCAAATGCCGTTGCGATCACAGATAG
TTCTTTTAAAAATGGTTTGATTAAAAGACAAGCTTTATCAGATGAGACTTGTCGTTTTGTGCCTTTTTTT
35 GGTCTAGCGAATGGAGTCGAATGGATAGTATGCACCCCTTCGGTGCTTGCGAGCGCTACAAGCGGAGCT
ATAGACCATTTTAAATTGGTAAGAGAGGATCAGCATCTTTGTGCGATTATTATGGTATACAACAAATTAC
CAATGAAATGCAAAAGAAAAAGCCATCTTTGTAGTATCTCCTCAATGGTTTACTGCTCAAGGGATTAAT
CCTAGTGCGGTTTCAAGTGTACTTGTCTAACACTCAAGTGATTGAATTTTACTAAAAGCTAGAACTGATA
AAGAATCACAGTTTGCAGCAAAGCGTTTGCTTGAGCTTAACCCTGGTGTGTCTAAATCAAACCTATTGAA
40 AAAAGTAAGTAAGGTAAGTCTCTTAGTTCGGTTAGACAGAGCTATTTTGAAATGTCAACATCAAGTAGCA
TTGAGAGAAGAGTCCCTTTTTAGTTTTTTAGGCAAATCTACTAACTATGAAAAAAGAAATTTGCTCGCG
TTAAGGGATTACCTAAAGTATTTTCGTATAAACAATTGAATGCATTAGCAACTAAGAGAGGCCAATTAGC
AACAACCAACAACCGTTTTGGGATTAAAAATACATTTTATCGTAAACGAATAGCACCTAAATACAATCTT
TATAAGAATTTCCAAGTTAATTATAGTTACCTGGCGTCACCAGAATACAATGATTTTCAGCTTTTATTAT
45 CAGAATTTGCTAAACGAAAAACAGATGTACTCTTTGTTATAACTCCTGTTAATAAAGCTTGGGCGGATTA
TACCGGCTTAAATCAAGATAAGTATCAAGCGGCAGTTCGTAAATAAAATTCAGTTAAAGTCACAAGGA
TTTCATCGCATTGCTGACTTCTCAAAAGATGGTGGTGAGTCTACTTTATGCAAGATACCATCCATCTCG
GTTGGAATGGCTGGTTAGCTTTTTGATAAGAAAGTGCAACCATTCTAGAAACGAAGCAGCCAGTGCCCAA
CTATAAAATGAACCCTTATTTTTATAGTAAATTTGGGCAAATAGGAAAGACTTGCAATAG

50

Preferred GAS 202 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 59; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 59, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 202 proteins include variants (e.g. allelic variants, homologs, orthologs, paralog, mutants, etc.) of SEQ ID NO: 59. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 59. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 59. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(31) GAS 057

GAS 057 corresponds to M1 GenBank accession numbers GI:13621655 and GI:15674549, to M3 GenBank accession number GI: 21909834, to M18 GenBank accession number GI: 19745560 and is also referred to as 'Spy0416' (M1), 'SpyM3_0298' (M3), 'SpyM18_0464' (M18) and 'prtS'. GAS 057 has also been identified as a putative cell envelope proteinase. Amino acid and polynucleotide sequences of GAS 057 of an M1 strain are set forth below:

SEQ ID NO: 61

MEKKQRFSLRKYKSGTFSVLIGSVFLVMTTVADELSTMSEPTITNHAQQQAQHLTNTELSSAESKSQD
TSQITLKTNRKEQSQDLVSEPTTTELADTDAASMANTGSDATQKSASLPPVNTDVHDWVKTKGAWDKGY
KGQGVVAVIDTGIDPAHQSMRISDVSTAKVKSKEMLARQKAAGINYGSWINDKVVFAHNYVENSNDIK
ENQFEDFDEWENFEFDAEAPKAIKKHKIYRQSTQAPKETVIKTEETDGSHDIDWTQTDDDTKYESHG
MHVTGIVAGNSKEAAATGERFLGIAPEAQVMFMRVVFANDIMGSAESLFKAIEDAVALGADVNLISLGT
NGAQLSGSKPLMEAEIEKAKKAGVSVVVAAGNERVYGSDDHDDPLATNPDYGLVGSPTSTGRTPTSVAAINSK
WVIQRLMTVKELENRADLNHGKAIYSESVDKDIKDSLGYDKSHQFAYVKESTDAGYNAQDVKGKIALIE
RDPNKTYDEMIALAKKHGALGVLI FNNKPGQSNRSMRLTANGMGI PSAFI SHEFGKAMSQNLNGNGTGSLE
FDSVVSKAPSQKGNEMNHFSNWGLTSDGYLKPDI TAPGGDIYSTYNDNHYGSQTGTSMAS PQIAGASLLV
KQYLEKTQPNLPKEKIADIVKNLLMSNAQIHVNPETKTTTSPRQOGAGLLNIDGAVTSGLYVTGKDNYS
ISLGNITDITMTFDVTVHNLNNDKTLRYDTELLTDHVDPOKGRFTLTSHSLKTYQGGEVTV PANGKVTVR
VTMDVSQFTKELTKQMPNGYYLEGFVFRDSDQDDQLNRVNI PFVGFPGQFENLAVAEESIYRLKSQKGTG
FYFDESGPKDDIYVGKHFTGLVTLGSETNVSTKTI SDNGLHTLGT PKNADGKFILEKNAQGNPVLAI SPN
GDNNQDFAAFKGVFLRKYQGLKASVYHASDKEHKNPLWVSPESFKGDKNFNSDIRFAKSTTLLGTAFSGK
SLTGAELPDGHYHYVVSYPDVVGAQRQEMTFDMILDRQKPVLSQATFDPETNRFKPEPLKDRGLAGVRK
DSVFYLERKDNKPVTITINDSYKYVSVEDNKT FVERQADGSFILPLDKAKLGDFYVMVEDFAGNVAIAKL
GDHLPQTLGKTPIKLKLTGDNVYQTKETLKNLEMTQSDTGLVTNQAQLAVVHRNQPSQLTKMNQDFFIS
PNEDGNKDFVAFKGLKNVYNDLTVNVYAKDDHQKQTPIWSSQAGASVSAIESTAWYGITARGSKVMPGD
YQYVVITYRDEHGKEHQKQYTI SVNDKKPMITQGRFDITINGVDHFTPDKTKALDSSGIVREEVFYLAKKNG
RKFDVTEGKDGITVSDNKVYI PKNPDGSYTI SKRDGVTLSDYIYLVEDRAGNVSFATLRDLKAVGKDKAV
VNFGLDLPVPEDKQIVNFTYLVRDADGKPIENLEYNNNSGNSLILPYGKYTVELLTYDTNAAKLES DKIV
SFTLSADNNFQQVTFKI TMLATSQITAHFDHLLPEGSRVSLKTAQDQLI PLEQSLYVPKAYGKTVQEGTY
EVVVSLPKGYRIEGNTKVNTLPNEVHELRLVKGVDASDSTGDHKVMSKNNSQALTASATPTKSTTSAT
AKALPSTGEKMGLKLRI VGLVLLGLTCVFSRKKSTKD

SEQ ID NO: 62

GTGGAGAAAAAGCAACGTTTTTCCCTTAGAAAAATACAAATCAGGAACGTTTTTCGGTCTTAATAGGAAGCG
TTTTCTTGGTGATGACAACAACAGTAGCAGCAGATGAGCTAAGCACAATGAGCGAACCAACAATCACGAA
TCACGCTCAACAACAAGCGCAACATCTCACCATAACAGAGTTGAGCTCAGCTGAATCAAAATCTCAAGAC
ACATCACAATCACTCTCAAGACAAATCGTGAAAAAGAGCAATCACAAGATCTAGTCTCTGAGCCAACCA
CAACTGAGCTAGCTGACACAGATGCAGCATCAATGGCTAATACAGGTTCTGATGCGACTCAAAAAGCGC
TTCTTTACCGCCAGTCAATACAGATGTTTACGATTGGGTAAAAACCAAGGAGCTTGGGACAAGGGATAC

AAAGGACAAGGCAAGGTTGTCGCAGTTATTGACACAGGGATCGATCCGGCCCATCAAAGCATGCGCATCA
 GTGATGTATCAACTGCTAAAGTAAATCAAAGAAGACATGCTAGCACGCCAAAAGCCGCGGTATTAA
 TTATGGGAGTTGGATAAATGATAAAGTTGTTTTGACATAATTATGTGGAAAATAGCGATAATATCAA
 GAAAATCAATTTCGAGGATTTTGATGAGGACTGGGAAAACCTTTGAGTTTGATGCAGAGGCAGAGCCAAAAG
 5 CCATCAAAAAACACAAGATCTATCGTCCCAATCAACCCAGGCACCGAAAGAACTGTTATCAAAACAGA
 AGAAACAGATGGTTCACATGATATTGACTGGACACAAACAGACGATGACACCAAATACGAGTCACACGGT
 ATGCATGTGACAGGTATTGTAGCCGGTAATAGCAAAGAAGCCGCTGCTACTGGAGAACGCTTTTTAGGAA
 TTGCACCAGAGGCCAAGTCATGTTTCATGCGTGTGTTTGGCCAACGACATCATGGGATCAGCTGAATCACT
 CTTTATCAAAGCTATCGAAGATGCCGTGGCTTTAGGAGCAGATGTGATCAACCTGAGTCTTGGAACCGCT
 10 AATGGGGCACAGCTTAGTGGCAGCAAGCCTCTAATGGAAGCAATTGAAAAAGCTAAAAAGCCGGTGTAT
 CAGTTGTTGTAGCAGCAGGAAATGAGCGCGTCTATGGATCTGACCATGATGATCCATTGGCGACAAATCC
 AGACTATGGTTTGGTCCGTTCTCCCTCAACAGGTGGAACACCAACATCAGTGGCAGCTATAAACAGTAAG
 TGGGTGATTCAACGTCTAATGACGGTCAAAGAATTAGAAAACCGTGCCGATTTAAACCATGGTAAAGCCA
 TCTATTGAGAGTCTGTGCACTTTAAAGACATAAAAGATAGCCTAGGTTATGATAAATCGCATCAATTTGC
 15 TTATGTCAAAGAGTCAACTGATGCGGGTTATAACGCACAAGACGTTAAAGGTAAAATTGCTTTAATTGAA
 CGTGATCCCAATAAAACCTATGACGAAATGATTGCTTTGGCTAAGAAACATGGAGCTCTGGGAGTACTTA
 TTTTAAATAACAAGCCTGGTCAATCAAACCGCTCAATGCGTCTAACAGCTAATGGGATGGGGATACCATC
 TGCTTTCATATCGCACGAATTTGGTAAGGCCATGTCCCAATTAATGGCAATGGTACAGGAAGTTTAGAG
 TTTGACAGTGTGGTCTCAAAGCACCGAGTCAAAGGCAATGAAATGAATCATTTTTCAAATTGGGGCC
 20 TAACCTCTGATGGCTATTTAAACCTGACATTACTGCACCAGGTGGCGATATCTATTCTACCTATAACGA
 TAACCACTATGGTAGCCAAACAGGAACAAGTATGGCCTCTCCTCAGATTGCTGGCGCCAGCCTTTTGCTC
 AAACAATACCTAGAAAAGACTCAGCCAAACTTGCCAAAAGAAAAAATTGCTGATATCGTTAAGAACCTAT
 TGATGAGCAATGCTCAAATTCATGTTAATCCAGAGACAAAACGACCACCTCACCGCGTCAGCAAGGGGC
 AGGATTACTTAATATTGACGGAGCTGTCACTAGCGGCCTTTATGTGACAGGAAAAGACAACCTATGGCAGT
 25 ATATCATTAGGCAACATCACAGATACGATGACGTTTGATGTGACTGTTCAACCTAAGCAATAAAGACA
 AAACATTACGTTATGACACAGAATTGCTAACAGATCATGTAGACCCACAAAAGGGCCGCTTCACTTTGAC
 TTCTCACTCCTTAAAAACGTACCAAGGAGGAGAAGTTACAGTCCAGCCAATGGAAAAGTGACTGTAAGG
 GTTACCATGGATGTCTCACAGTTCACAAAAGAGCTAACAAAACAGATGCCAAATGGTTACTATCTAGAAG
 GTTTTGTCCGCTTTAGAGATAGTCAAGATGACCAACTAAATAGAGTAAACATTCTTTTGTGGTTTAA
 30 AGGGCAATTTGAAAACCTAGCAGTTGCAGAAGAGTCCATTTACAGATTAAATCTCAAGGCAAACTGGT
 TTTTACTTTGATGAATCAGGTCCAAAAGACGATATCTATGTCCGTAAACACTTTACAGGACTTGTCCTC
 TTGGTTTCAGAGACCAATGTGTCAACCAAAACGATTTCTGACAAATGGTCTACACACACTTGGCACCTTTAA
 AAATGCAGATGGCAAATTTATCTTAGAAAAAATGCCAAGGAAACCTGTCTTAGCCATTTCTCCAAAT
 GGTGACAACAACCAAGATTTTGCAGCCTTCAAAGGTGTTTCTTGAGAAAATATCAAGGCTTAAAGCAA
 35 GTGTCTACCATGCTAGTGACAAGGAACACAAAATCCACTGTGGGTGAGCCAGAAAGCTTTAAAGGAGA
 TAAAAACCTTAAATAGTGACATTAGATTGCAAAATCAACGACCCTGTTAGGCACAGCATTTTCTGGAAAA
 TCGTTAACAGGAGCTGAATTACCAGATGGGCATTATCATTATGTGGTGTCTTATTACCCAGATGTGGTCTG
 GTGCCAAACGTCAAGAAATGACATTTGACATGATTTTAGACCGACAAAACCGGTACTATCACAAGCAAC
 ATTTGATCCTGAAACAAACCGATTCAAACCAGAACCCCTAAAAGACCGTGGATTAGCTGGTGTTCGCAA
 40 GACAGTGTCTTTTATCTAGAAAAGAAAGACAACAAGCCTTATACAGTTACGATAAACGATAGCTACAAAT
 ATGTCTCAGTAGAAGACAATAAAACATTTGTGGAGCGACAAGCTGATGGCAGCTTTATCTTGCCGCTTGA
 TAAAGCAAAATTAGGGGATTTCTATTACATGGTCTGAGGATTTTGCAGGGAACGTGGCCATCGCTAAGTTA
 GGAGATCACTTACCACAAACATTAGGTAAAACACCAATTAACTTAAGCTTACAGACGGTAATTATCAGA
 CCAAAGAAACGCTTAAAGATAATCTTGAAATGACACAGTCTGACACAGGTCTAGTCACAAATCAAGCCCA
 45 GCTAGCAGTGGTGCACCGCAATCAGCCGCAAAGCCAGCTAACAAAGATGAATCAGGATTTCTTTATCTCA
 CCAAACGAAGATGGGAATAAAGACTTTGTGGCCTTTAAAGGCTTGAAAAATAACGTGTATAATGACTTAA
 CGGTTAACGTATACGCTAAAGATGACCACCAAAAACAAACCCCTATCTGGTCTAGTCAAGCAGGCGCTAG
 TGTATCCGCTATTGAAAGTACAGCCTGGTATGGCATAACAGCCCGAGGAAGCAAGGTGATGCCAGGTGAT
 TATCAGTATGTTGTGACCTATCGTGACGAACATGGTAAAGAACATCAAAGCAGTACACCATATCTGTGA
 50 ATGACAAAAACCAATGATCACTCAGGGACGTTTGTATACCATTAAATGGCGTTGACCACTTTACTCCTGA
 CAAGACAAAAGCCCTTGACTCATCAGGCATTGTCCGCGAAGAAGTCTTTTACTTGGCCAAGAAAAATGGC
 CGTAAATTTGATGTGACAGAAGGTAAAGATGGTATCACAGTTAGTGACAATAAGGTGTATATCCCTAAAA
 ATCCAGATGGTTCTTACACCATTTCAAAGAGATGGTGTCACTGTGAGATTATTACTACCTTGTCTGA
 AGATAGAGCTGGTAATGTGTCTTTTGTCTACCTTGCGTGACCTAAAAGCGGTGCGAAAAGACAAAGCAGTA
 55 GTCAATTTGGATTAGACTTACCGGTCCCTGAAGACAAACAAATAGTGAACTTTACCTACCTTGTGCGGG
 ATGCAGATGGTAAACCGATTGAAAACCTAGAGTATTATAATAACTCAGGTAACAGTCTTATCTTGCCATA
 CGGCAAATACACGGTCAATTTGTTGACCTATGACACCAATGCAGCCAACTAGAGTCAGATAAAATCGTT
 TCCTTTACCTTGTGAGCTGATAACAACCTTCCAACAAGTTACCTTTAAGATAACGATGTTAGCAACTTCTC
 AAATAACTGCCCACTTTGATCATCTTTTGGCAGAAGGCAGTCGCGTTAGCCTTAAACAGCTCAAGATCA
 60 GCTAATCCCGCTTGAACAGTCCTTGTATGTGCCTAAAGCTTATGGCAAACCGTTCAAGAAGGCCTTAC
 GAAGTTGTGTGACGCTGCCTAAAGGCTACCGTATCGAAGGCAACACAAAGGTGAATACCTACCAATG
 AAGTGCACGAATCATATTACGCTTGTCAAAGTAGGAGATGCCTCAGATTCAACTGGTGATCATAAGGT

TATGTCAAAAAATAATTCACAGGCTTTGACAGCCTCTGCCACACCAACCAAGTCAACGACCTCAGCAACA
GCAAAAGCCCTACCATCAACGGGTGAAAAAATGGGTCTCAAGTTGCGCATAGTAGGTCTTGTGTTACTCG
GACTTACTTGCGTCTTTAGCCGAAAAAATCAACCAAAGATTGA

5 Preferred GAS 057 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 61; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 61, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). These GAS 057 proteins include variants (e.g.
10 allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 61. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 61. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 61. For example, in one embodiment, the underlined amino acid sequence at the N-terminus of
15 SEQ ID NO: 61 is removed. In another example, the underlined amino acid sequence at the C-terminus of SEQ ID NO: 61 is removed. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

The immunogenicity of other known GAS antigens may be improved by combination with two or
20 more GAS the first antigen group. Such other known GAS antigens include a second antigen group consisting of (1) one or more variants of the M surface protein or fragments thereof, (2) fibronectin-binding protein, (3) streptococcal heme-associated protein, or (4) SagA. These antigens are referred to herein as the "second antigen group".

The invention thus includes an immunogenic composition comprising a combination of GAS
25 antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group and one, two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and
30 GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

Each of the GAS antigens of the second antigen group are described in more detail below.

(1) M surface protein

Over 100 different type variants of the M protein have been identified. Epitopes having increased
35 bactericidal activity and having decreased likelihood of cross-reacting with human tissues have been identified in the amino terminal region and combined into fusion proteins containing approximately six, seven, or eight M protein fragments linked in tandem. See Ref. 4, 5, 6, WO 02/094851 and WO 94/06465. (Each of the M protein variants, fragments and fusion proteins described in these references are specifically incorporated herein by reference.)

Accordingly, the compositions of the invention may further comprise a GAS M surface protein or a fragment or derivative thereof. One or more GAS M surface protein fragments may be combined together in a fusion protein. Alternatively, one or more GAS M surface protein fragments are combined with a GAS antigen or fragment thereof of the first antigen group. One example of a GAS

5 M protein is set forth below.

SEQ ID NO: 63

MAKNNTNRHYSRLKLTGTASVAVALTVLGAGFANQTEVKANGDGNPREVIEDLAANNPAIQNIRLRYEN
KDLKARLENAMEVAGRDFKRAEELEKAKQALEQDKDLETKLKELQQDYDLAKESTSWDRQRLEKELEEK
KEALELAIDQASRDYHRATALEKELEEKKALELAIDQASQDYNRANVLEKELETITREQEINRNLLGNA
10 KLELDQLSSEKEQLTIEKAKLEEEKQISDASRQSLRRDLASREAKKQVEKDLANLTAEKDKVKEDKQIS
DASRQGLRRDLASREAKKQVEKDLANLTAEKDKVKEEKQISDASRQGLRRDLASREAKKQVEKALEEA
NSKLALEKLNKELEESKKLTEKEKAEKQAKLEABAKALKEQLAKQAEELAKLRAGKASDSQTPDTKPGN
KAVPGKGQAPQAGTKPNQNKAPMKETKRQLPSTGETANPFFTAAALTMATAGVAAVVKRKEEN

15 Preferred GAS M proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 63; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 63, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150 or more). These GAS M proteins include variants (e.g. allelic
20 variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 63. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 63. Preferably, the fragment is one of those described in the references above. Preferably, the fragment is constructed in a fusion protein with one or more additional M protein fragments. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2,
25 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 63. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(2) Fibronectin-binding protein

GAS fibronectin-binding protein ('Sfbl') is a multifunctional bacterial protein thought to mediate
30 attachment of the bacteria to host cells, facilitate bacterial internalization into cells and to bind to the Fc fragment of human IgG, thus interfering with Fc-receptor mediated phagocytosis and antibody-dependent cell cytotoxicity. Immunization of mice with Sfbl and an 'H12 fragment' (encoded by positions 1240 – 1854 of the Sfbl gene) are discussed in Refs. 7,8 and 9. One example of an amino acid sequence for GAS Sfbl is show below.

35 **SEQ ID NO: 64**

MSFDGFFLHHLTNELKENLLYGRIQKVNQPFERELVLTIRNHRKNYKLLLSAHPVFGRVQITQADFQNPQ
VPNTFTMIMRKYLQGAVIEQLEQIDNDRIIEIKVSNKNEIGDAIQATLIIIEIMGKHSNIIILVDRAENKII
ESIKHVGFSONSYRTILPGSTYIEPPKTAAVNPFTITDVPLFEILQTQELTVKSLQQHFQGLGRDTAKEL
AELLTTDKLKRFRBFFARPTQANLTASFAPVLFSDSHATFETLSMDLDFYQDKAERDRINQQASDLIH
40 RVQTELDKNRNKLKQAEELLATENAELFRQKGELLTTYLSLVFNQDSVILDNYTGEKIEIALDKALT
PNQNAQRYFKKYQKLKEAVKHLGLIADTKQSITYFESVDYNLSQASIDDIREDIYQAGFLKSRQRD
KRHKRKKPEQYLASDGTITLMVGRNNLQNEELTFKMAKKGELWPHAKDIPGSHVVIKDNLDPSDEVKTD
AELAAYYSKARLSNLVQVDMIEAKKLHKPSGAKPGFVITYTGOKTLRVTDPQAKILSMKLS

Preferred SfbI proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 64; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 64, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, or more). These SfbI proteins include variants (e.g. allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 64. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 64. Preferably, the fragment is one of those described in the references above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 64. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(3) *Streptococcal heme-associated protein*

The GAS streptococcal heme-associated protein ('Shp') has been identified as a GAS cell surface protein. It is thought to be cotranscribed with genes encoding homologues of an ABC transporter involved in iron uptake in gram-negative bacteria. The Shp protein is further described in 10. One example of a Shp protein is shown below:

SEQ ID NO: 65

MTKVVIKQLLQVIVVFMISLSTMTNLVYADKGQIYGCI IQRNYRHPI SGQIEDSGGEHSFDIGQGMVEGT
VYSDAMLEVSDAGKIVLTFRMSLADYSGNYQFWIQPGGTGSFQAVDYNITQKGTDTNGTTLDAISLPTV
NSIIRGSMFVEPMGREVVFYLSASELIQKYSGNMLAQLVTETDNSQNQEVKDSQKPVDTKLGESQDESHT
GAMITQNKPKANSSNNKSLSDKKILPSKMGLTTSLELKKEDKFRSKKDL SIMIYYFPTFFLMLGGFAVWV
WKKRKKNDKTM

Preferred Shp proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 65; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 65, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). These Shp proteins include variants (e.g. allelic variants, homologs, orthologs, paralog, mutants, *etc.*) of SEQ ID NO: 65. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 65. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 65. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

(4) *SagA*

Streptolysin S (SLS), also known as 'SagA', is thought to be produced by almost all GAS colonies. This cytolytic toxin is responsible for the beta-hemolysis surrounding colonies of GAS grown on blood agar and is thought to be associated with virulence. While the full SagA peptide has not been

shown to be immunogenic, a fragment of amino acids 10 – 30 (SagA 10 – 30) has been used to produce neutralizing antibodies. See Ref. 11. The amino acid sequence of SagA 10 – 30 is shown below:

SEQ ID NO: 66 FSIATGSGNSQGGSGSYTPGKC

- 5 Preferred SagA 10-30 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 66; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 66, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, or 20). These SagA 10 - 30 proteins include variants (e.g. allelic variants, homologs, orthologs, 10 paralogs, mutants, *etc.*) of SEQ ID NO: 66.

There is an upper limit to the number of GAS antigens which will be in the compositions of the invention. Preferably, the number of GAS antigens in a composition of the invention is less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, 15 or less than 3. Still more preferably, the number of GAS antigens in a composition of the invention is less than 6, less than 5, or less than 4. Still more preferably, the number of GAS antigens in a composition of the invention is 3.

The GAS antigens used in the invention are preferably isolated, i.e., separate and discrete, from the whole organism with which the molecule is found in nature or, when the polynucleotide or 20 polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

Fusion proteins

The GAS antigens used in the invention may be present in the composition as individual separate polypeptides, but it is preferred that at least two (*i.e.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 25 18, 19 or 20) of the antigens are expressed as a single polypeptide chain (a 'hybrid' polypeptide). Hybrid polypeptides offer two principal advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

30 The hybrid polypeptide may comprise two or more polypeptide sequences from the first antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a GAS antigen or a fragment thereof of the first antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes.

35 The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the second antigen group. Accordingly, the invention

includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a GAS antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a GAS antigen or a fragment thereof from the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes:

Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten GAS antigens are preferred. In particular, hybrids consisting of amino acid sequences from two, three, four, or five GAS antigens are preferred.

Different hybrid polypeptides may be mixed together in a single formulation. Within such combinations, a GAS antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Hybrid polypeptides can be represented by the formula $\text{NH}_2\text{-A-}\{-\text{X-L-}\}_n\text{-B-COOH}$, wherein: X is an amino acid sequence of a GAS antigen or a fragment thereof from the first antigen group or the second antigen group; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and n is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of $X_2 \dots X_n$ will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each n instances of $\{-\text{X-L-}\}$, linker amino acid sequence -L- may be present or absent. For instance, when $n=2$ the hybrid may be $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-L}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-X}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-L}_1\text{-X}_2\text{-COOH}$, $\text{NH}_2\text{-X}_1\text{-X}_2\text{-L}_2\text{-COOH}$, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where $n = 2, 3, 4, 5, 6, 7, 8, 9, 10$ or more), and histidine tags (*i.e.* His_n where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG, with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(\text{Gly})_4$ tetrapeptide being a typical poly-glycine linker.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more). Other suitable N-terminal amino acid sequences will be.

apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (e.g. with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (e.g. 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (e.g. comprising histidine tags *i.e.* His_{*n*}, where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Most preferably, *n* is 2 or 3.

10 The invention also provides nucleic acid encoding hybrid polypeptides of the invention. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (e.g. 65°C in a 0.1xSSC, 0.5% SDS solution).

Polypeptides of the invention can be prepared by various means (e.g. recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (e.g. native, fusions, 15 non-glycosylated, lipidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other GAS or host cell proteins).

Nucleic acid according to the invention can be prepared in many ways (e.g. by chemical synthesis, from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (e.g. single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in substantially 20 pure form (*i.e.* substantially free from other GAS or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (e.g. phosphorothioates, *etc.*), and also peptide nucleic acids (PNA), *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (e.g. for antisense or probing purposes).

25 The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

30 The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (e.g. PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

Strains

Preferred polypeptides of the invention comprise an amino acid sequence found in an M1, M3 or M18 strain of GAS. The genomic sequence of an M1 GAS strain is reported at Ref. 12. The genomic sequence of an M3 GAS strain is reported at Ref. 13. The genomic sequence of an M18 GAS strain is reported at Ref. 14.

Where hybrid polypeptides are used, the individual antigens within the hybrid (i.e. individual -X-moieties) may be from one or more strains. Where $n=2$, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where $n=3$, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2 \neq X_3$ (iii) $X_1 \neq X_2 = X_3$ (iv) $X_1 \neq X_2 \neq X_3$ or (v) $X_1 = X_3 \neq X_2$, etc.

10 **Purification and Recombinant Expression**

The GAS antigens of the invention may be isolated from a *Streptococcus pyogenes*, or they may be recombinantly produced, for instance, in a heterologous host. Preferably, the GAS antigens are prepared using a heterologous host. The heterologous host may be prokaryotic (e.g. a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*, *Vibrio cholerae*,
15 *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (e.g. *M.tuberculosis*), yeasts, etc.

Recombinant production of polypeptides is facilitated by adding a tag protein to the GAS antigen to be expressed as a fusion protein comprising the tag protein and the GAS antigen. Such tag proteins can facilitate purification, detection and stability of the expressed protein. Tag proteins suitable for
20 use in the invention include a polyarginine tag (Arg-tag), polyhistidine tag (His-tag), FLAG-tag, Strep-tag, c-myc-tag, S-tag, calmodulin-binding peptide, cellulose-binding domain, SBP-tag, chitin-binding domain, glutathione S-transferase-tag (GST), maltose-binding protein, transcription termination anti-terminiation factor (NusA), *E. coli* thioredoxin (TrxA) and protein disulfide isomerase I (DsbA). Preferred tag proteins include His-tag and GST. A full discussion on the use of
25 tag proteins can be found at Ref. 15.

After purification, the tag proteins may optionally be removed from the expressed fusion protein, i.e., by specifically tailored enzymatic treatments known in the art. Commonly used proteases include enterokinase, tobacco etch virus (TEV), thrombin, and factor X_a .

Immunogenic compositions and medicaments

30 Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 8, preferably about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen-free. The composition may be isotonic with respect to humans.

Vaccines according to the invention may either be prophylactic (i.e. to prevent infection) or
35 therapeutic (i.e. to treat infection), but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of a *Streptococcus pyogenes* infection

in an animal susceptible to streptococcal infection comprising administering to said animal a therapeutic or prophylactic amount of the immunogenic compositions of the invention. Preferably, the immunogenic composition comprises a combination of GAS antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group. Preferably, the combination of GAS
5 antigens consists of three, four, five, six, seven, eight, nine, or ten GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens consists of three, four, or five GAS antigens selected from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117.

Alternatively, the invention includes an immunogenic composition comprising a combination of GAS
10 antigens, said combination consisting of two to thirty-one GAS antigens of the first antigen group and one, two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and
15 GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

20 The invention also provides the use of the compositions of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides for a kit comprising a first component comprising a combination of GAS antigens. In one embodiment, the combination of GAS antigens consists of a mixture of two to thirty-one GAS antigens selected from the first antigen group. Preferably, the combination consists of three,
25 four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Preferably, the combination consists of three, four, or five GAS antigens from the first antigen group. Preferably, the combination includes either or both of GAS 117 and GAS 040.

In another embodiment, the kit comprises a first component comprising a combination of GAS antigens consisting of a mixture of two to thirty-one GAS antigens of the first antigen group and one,
30 two, three, or four GAS antigens of the second antigen group. Preferably, the combination consists of three, four, five, six, seven, eight, nine, or ten GAS antigens from the first antigen group. Still more preferably, the combination consists of three, four or five GAS antigens from the first antigen group. Preferably, the combination of GAS antigens includes either or both of GAS 40 and GAS 117. Preferably, the combination of GAS antigens includes one or more variants of the M surface protein.

35 The invention also provides a delivery device pre-filled with the immunogenic compositions of the invention.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies and/or cell-mediated immunity. The method may raise a booster response.

- 5 The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (e.g. a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults e.g. to assess safety, dosage, immunogenicity, etc.

- 10 These uses and methods are preferably for the prevention and/or treatment of a disease caused by *Streptococcus pyogenes* (e.g. pharyngitis (such as streptococcal sore throat), scarlet fever, impetigo, erysipelas, cellulitis, septicemia, toxic shock syndrome, necrotizing fasciitis (flesh eating disease) and sequelae (such as rheumatic fever and acute glomerulonephritis)). The compositions may also be effective against other streptococcal bacteria.

- 15 One way of checking efficacy of therapeutic treatment involves monitoring GAS infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the GAS antigens in the compositions of the invention after administration of the composition.

- 20 Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (e.g. subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral (e.g. tablet, spray), vaginal, topical, transdermal {e.g. see ref. 16} or transcutaneous {e.g. see refs. 17 & 18}, intranasal {e.g. see ref. 19}, ocular, aural, pulmonary or other mucosal administration.

The invention may be used to elicit systemic and/or mucosal immunity.

- 25 Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes e.g. a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, etc.

- 30 The compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (e.g. a lyophilised composition). The composition may be prepared for topical administration e.g. as an ointment, cream or powder. The composition may be prepared for oral administration e.g. as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration e.g. as an inhaler, using a fine powder or a spray. The composition may be prepared as
35 a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration e.g. as drops. The composition may be in kit form, designed such that a combined composition is

reconstituted just prior to administration to a patient. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (e.g. non-human primate, primate, etc.), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Further components of the composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition.

Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in reference 20.

Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant.

Preferred further adjuvants include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (e.g. oxyhydroxides), phosphates (e.g. hydroxyphosphates, orthophosphates), sulphates, etc. (e.g. see chapters 8 & 9 of ref. 21)), or mixtures of different mineral compounds, with the compounds taking any suitable form (e.g. gel, crystalline, amorphous, etc.); and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt. See ref. 22.

B. Oil-Emulsions

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See ref. 23.

Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

C. Saponin Formulations

Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsapilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs.

Saponin compositions have been purified using High Performance Thin Layer Chromatography (HPLC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in U.S. Patent No. 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO 96/33739).

Combinations of saponins and cholesterol can be used to form unique particles called Immunostimulating Complexs (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP 0 109 942, WO 96/11711 and WO 96/33739. Optionally, the ISCOMS may be devoid of additional detergent. See ref. 24.

A review of the development of saponin based adjuvants can be found at ref. 25.

C. Virosomes and Virus Like Particles (VLPs)

Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, QB-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO 03/024480, WO 03/024481, and Refs. 26, 27, 28 and 29. Virosomes are discussed further in, for example, Ref. 30

D. Bacterial or Microbial Derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

- (1) *Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)*

Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL).

3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives e.g. RC-529. See Ref. 31.

(2) *Lipid A Derivatives*

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in Ref. 32 and 33.

(3) *Immunostimulatory oligonucleotides*

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogues such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analog such as 2'-deoxy-7-deazaguanosine. See ref. 34, WO 02/26757 and WO 99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Refs. 35, 36, WO 98/40100, U.S. Patent No. 6,207,646, U.S. Patent No. 6,239,116, and U.S. Patent No. 6,429,199.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See ref. 37. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such as a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 38, 39 and WO 01/95935. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 40, 41, 42 and WO 03/035836.

(4) *ADP-ribosylating toxins and detoxified derivatives thereof.*

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (i.e., *E. coli* heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO 95/17211 and as parenteral adjuvants in WO 98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63.

E. Human Immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g. interferon- γ), macrophage colony stimulating factor, and tumor necrosis factor.

F. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Ref. 43) or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrrolidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. E.g., ref. 44.

G. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (i.e. a particle of ~100nm to ~150µm in diameter, more preferably ~200nm to ~30µm in diameter, and most preferably ~500nm to ~10µm in diameter) formed from materials that are biodegradable and non-toxic (e.g. a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, etc.), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (e.g. with SDS) or a positively-charged surface (e.g. with a cationic detergent, such as CTAB).

H. Liposomes

Examples of liposome formulations suitable for use as adjuvants are described in U.S. Patent No. 6,090,406, U.S. Patent No. 5,916,588, and EP 0 626 169.

I. Polyoxyethylene ether and Polyoxyethylene Ester Formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. Ref. 45. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (Ref. 46) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (Ref. 47).

Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in Ref. 48 and 49.

K. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetylmuramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinolone Compounds

Examples of imidazoquinolone compounds suitable for use as adjuvants in the invention include Imiquimod and its homologues, described further in Ref. 50 and 51.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

- (1) a saponin and an oil-in-water emulsion (ref. 52);

(2) a saponin (e.g., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) (see WO 94/00153);

(3) a saponin (e.g., QS21) + a non-toxic LPS derivative (e.g., 3dMPL) + a cholesterol;

(4) a saponin (e.g. QS21) + 3dMPL + IL-12 (optionally + a sterol) (Ref. 53);

5 combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (Ref. 54);

(5) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(6) RibiTM adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DetoxTM); and

(7) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

15 Aluminium salts and MF59 are preferred adjuvants for parenteral immunisation. Mutant bacterial toxins are preferred mucosal adjuvants.

The composition may include an antibiotic.

Further antigens

The compositions of the invention may further comprise one or more additional non-GAS antigens, including additional bacterial, viral or parasitic antigens.

In one embodiment, the GAS antigen combinations of the invention are combined with one or more additional, non-GAS antigens suitable for use in a paediatric vaccine. For example, the GAS antigen combinations may be combined with one or more antigens derived from a bacteria or virus selected from the group consisting of *N. meningitidis* (including serogroup A, B, C, W135 and/or Y),

25 *Streptococcus pneumoniae*, *Bordetella pertussis*, *Moraxella catarrhalis*, *Tetanus*, *Diphtheria*, Respiratory Syncytial virus ('RSV'), polio, measles, mumps, rubella, and rotavirus.

In another embodiment, the GAS antigen combinations of the invention are combined with one or more additional, non-GAS antigens suitable for use in a vaccine designed to protect elderly or immunocomprised individuals. For example, the GAS antigen combinations may be combined

30 with an antigen derived from the group consisting of *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Listeria monocytogenes*, influenza, and Parainfluenza virus ('PIV').

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity {e.g. refs. 55 to 64}. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM₁₉₇ diphtheria toxoid is particularly

35 preferred {65}. Other carrier polypeptides include the *N.meningitidis* outer membrane protein {66},

synthetic peptides {67, 68}, heat shock proteins {69, 70}, pertussis proteins {71, 72}, protein D from *H.influenzae* {73}, cytokines {74}, lymphokines, hormones, growth factors, toxin A or B from *C.difficile* {75}, iron-uptake proteins {76}, etc. Where a mixture comprises capsular saccharides from both serogroups A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (e.g. 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary e.g. detoxification of pertussis toxin by chemical and/or genetic means.

- 10 Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

15 Antigens in the composition will typically be present at a concentration of at least 1 µg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used (e.g. refs. 77 to 85). Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA e.g. in the form of a plasmid) that encodes the protein.

Definitions

The term "comprising" means "including" as well as "consisting" e.g. a composition "comprising" X may consist exclusively of X or may include something additional e.g. X + Y.

The term "about" in relation to a numerical value x means, for example, $x \pm 10\%$.

- 25 References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 86. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in reference 87.

30 The following example demonstrates one way of preparing recombinant GAS antigens of the invention and testing their efficacy in a murine model.

EXAMPLE 1: Preparation of recombinant GAS antigens of the invention and Demonstration of Efficacy in Murine Model.

Recombinant GAS proteins corresponding to two or more of the GAS antigens of the first antigen group are expressed as follows.

1. Cloning of GAS antigens for expression in E. coli

5 The selected GAS antigens were cloned in such a way to obtain two different kinds of recombinant proteins: (1) proteins having an hexa-histidine tag at the carboxy-terminus (Gas-His) and (2) proteins having the hexa-histidine tag at the carboxy-terminus and GST at the amino-terminus (Gst-Gas-His). Type (1) proteins were obtained by cloning in a pET21b+vector (available from Novagen). The type (2) proteins were obtained by cloning in a pGEX-NNH
10 vector. This cloning strategy allowed for the GAS genomic DNA to be used to amplify the selected genes by PCR, to perform a single restriction enzyme digestion of the PCR products and to clone then simultaneously into both vectors.

(a) Construction of pGEX-NNH expression vectors

Two couples of complementary oligodeoxyribonucleotides are synthesised using the DNA synthesiser
15 ABI394 (Perkin Elmer) and reagents from Cruachem (Glasgow, Scotland). Equimolar amounts of the
oligo pairs (50 ng each oligo) are annealed in T4 DNA ligase buffer (New England Biolabs) for 10
min in a final volume of 50 μ l and then left to cool slowly at room temperature. With the described
procedure the following DNA linkers are obtained:

gexNN linker

20 NdeI NheI XmaI EcoRI NcoI SalI XhoI SacI
GATCCCATATGGCTAGCCCGGGGAATTCGTCCATGGAGTGAGTCGACTGACTCGAGTGATCGAGCTC
GGTATACCGATCGGGCCCCTTAAGCAGGTACCTCACTCAGCTGACTGAGCTCACTAGCTCGAG

Not I

25 CTGAGCGGCCGCATGAA
GACTCGCCGGCGTACTTTCGA

gexNNH linker

30 HindIII NotI XhoI Hexa-Histidine
TCGACAAGCTTGGCGCCGCACTCGAGCATCACCATCACCATCACTGAT
GTTCGAACGCCGCGGTGAGCACGTAGAGGTAGTGGTAGTGACTATCGA

The plasmid pGEX-KG [K. L. Guan and J. E. Dixon, *Anal. Biochem.* 192, 262 (1991)] is digested with BamHI and HindIII and 100 ng is ligated overnight at 16 °C to the linker gexNN with a molar ratio of 3:1 linker/plasmid using 200 units of T4 DNA ligase (New england Biolabs). After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NN plasmid, having the correct linker, is selected by means of restriction enzyme analysis and DNA sequencing.

The new plasmid pGEX-NN is digested with Sall and HindIII and ligated to the linker gexNNH. After transformation of the ligation product in *E. coli* DH5, a clone containing the pGEX-NNH plasmid, having the correct linker, is selected by means of restriction enzyme analysis and DNA sequencing.

(b) *Chromosomal DNA preparation*

5 GAS SF370 strain is grown in THY medium until OD₆₀₀ is 0.6-0.8. Bacteria are then centrifuged, suspended in TES buffer with lysozyme (10mg/ml) and mutanolysine (10U/μl) and incubated 1 hr at 37° C. Following treatment of the bacterial suspension with RNAase, Proteinase K and 10% Sarcosyl/EDTA, protein extraction with saturated phenol and phenol/chloroform is carried out. The resulting supernatant is precipitated with Sodium Acetate/Ethanol and the extracted DNA is pelleted
10 by centrifugation, suspended in Tris buffer and kept at -20° C.

(c) *Oligonucleotide design*

Synthetic oligonucleotide primers are designed on the basis of the coding sequence of each GAS antigen using the sequence of *Streptococcus pyogenes* SF370 M1 strain. Any predicted signal peptide is omitted, by deducing the 5' end amplification primer sequence immediately downstream from the
15 predicted leader sequence. For most GAS antigens, the 5' tail of the primers (see Table 1, below) include only one restriction enzyme recognition site (NdeI, or NheI, or SpeI depending on the gene's own restriction pattern); the 3' primer tails (see Table 1) include a XhoI or a NotI or a HindIII restriction site.

5' tails		3' tails	
NdeI	5' GTGCGTCATATG 3'	XhoI	5' GCGTCTCGAG 3'
NheI	5' GTGCGTGCTAGC 3'	NotI	5' ACTCGCTAGCGGCCGC 3'
SpeI	5' GTGCGTACTAGT 3'	HindIII	5' GCGTAAGCTT 3'

20 Table 1. Oligonucleotide tails of the primers used to amplify genes encoding selected GAS antigens.

As well as containing the restriction enzyme recognition sequences, the primers include nucleotides which hybridize to the sequence to be amplified. The number of hybridizing nucleotides depends on the melting temperature of the primers which can be determined as described [(Breslauer et al., Proc. Nat. Acad. Sci. 83, 3746-50 (1986))]. The average melting temperature of the selected oligos is 50-55
25 °C for the hybridizing region alone and 65-75 °C for the whole oligos. Oligos can be purchased from MWG-Biotech S.p.A. (Firenze, Italy).

(d) *PCR amplification*

The standard PCR protocol is as follows: 50 ng genomic DNA are used as template in the presence of 0,2 μM each primer, 200 μM each dNTP, 1,5 mM MgCl₂, 1x PCR buffer minus Mg (Gibco-BRL),
30 and 2 units of Taq DNA polymerase (Platinum Taq, Gibco-BRL) in a final volume of 100 μl. Each sample undergoes a double-step amplification: the first 5 cycles are performed using as the hybridizing temperature of one of the oligos excluding the restriction enzyme tail, followed by 25

cycles performed according to the hybridization temperature of the whole length primers. The standard cycles are as follows:

one cycle:

denaturation : 94 °C, 2 min

5

5 cycles:

denaturation: 94 °C, 30 seconds, hybridization: } 1 °C, 50 seconds, elongation: 72 °C, 1 min or
2 min and 40 sec

10

25 cycles:

denaturation: 94 °C, 30 seconds

hybridization: 70 °C, 50 seconds

elongation: 72 °C, 1 min or 2 min and 40 sec }

15

72 °C, 7 min

4 °C

The elongation time is 1 min for GAS antigens encoded by ORFs shorter than 2000 bp, and 2 min and 40 seconds for ORFs longer than 2000 bp. The amplifications are performed using a Gene Amp PCR system 9600 (Perkin Elmer).

20

To check the amplification results, 4 µl of each PCR product is loaded onto 1-1.5 agarose gel and the size of amplified fragments compared with DNA molecular weight standards (DNA markers III or IX, Roche). The PCR products are loaded on agarose gel and after electrophoresis the right size bands are excised from the gel. The DNA is purified from the agarose using the Gel Extraction Kit (Qiagen) following the instruction of the manufacturer. The final elution volume of the DNA is 50 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8). One µl of each purified DNA is loaded onto agarose gel to evaluate the yield.

25

(e) *Digestion of PCR fragments*

One-two µg of purified PCR products are double digested overnight at 37 °C with the appropriate restriction enzymes (60 units of each enzyme) using the appropriate restriction buffer in 100 µl final volume. The restriction enzymes and the digestion buffers are from New England Biolabs. After purification of the digested DNA (PCR purification Kit, Qiagen) and elution with 30 µl TE, 1 µl is subjected to agarose gel electrophoresis to evaluate the yield in comparison to titrated molecular weight standards (DNA markers III or IX, Roche).

30

(f) *Digestion of the cloning vectors (pET21b+ and pGEX-NNH)*

10 µg of plasmid is double digested with 100 units of each restriction enzyme in 400 µl reaction volume in the presence of appropriate buffer by overnight incubation at 37 °C. After electrophoresis on a 1% agarose gel, the band corresponding to the digested vector is purified from the gel using the Qiagen Qiaex II Gel Extraction Kit and the DNA was eluted with 50 µl TE. The DNA concentration is evaluated by measuring OD₂₆₀ of the sample.

35

(g) *Cloning of the PCR products*

Seventy five ng of the appropriately digested and purified vectors and the digested and purified fragments corresponding to each selected GAS antigen are ligated in final volumes of 10-20 μ l with a molar ratio of 1:1 fragment/vector, using 400 units T4 DNA ligase (New England Biolabs) in the presence of the buffer supplied by the manufacturer. The reactions are incubated overnight at 16 °C. Transformation of *E coli* BL21 (Novagen) and *E coli* BL21-DE3 (Novagen) electrocompetent cells is performed using pGEX-NNH ligations and pET21b+ ligations respectively. The transformation procedure is as follows: 1-2 μ l the ligation reaction is mixed with 50 μ l of ice cold competent cells; then the cells are poured in a gene pulser 0.1 cm electrode cuvette (Biorad). After pulsing the cells in a MicroPulser electroporator (Biorad) following the manufacturer instructions the cells are suspended in 0.95 ml of SOC medium and incubated for 45 min at 37 °C under shaking. 100 and 900 μ l of cell suspensions are plated on separate plates of agar LB 100 μ g/ml Ampicillin and the plates are incubated overnight at 37 °C. The screening of the transformants is done by PCR: randomly chosen transformants are picked and suspended in 30 μ l of PCR reaction mix containing the PCR buffer, the 4 dNTPs, 1,5 mM MgCl₂, Taq polymerase and appropriate forward and reverse oligonucleotide primers that are able to hybridize upstream and downstream from the polylinker of pET21b+ or pGEX-NNH vectors. After 30 cycles of PCR, 5 μ l of the resulting products are run on agarose gel electrophoresis in order to select for positive clones from which the expected PCR band is obtained. PCR positive clones are chosen on the basis of the correct size of the PCR product, as evaluated by comparison with appropriate molecular weight markers (DNA markers III or IX, Roche).

2. Protein expression

PCR positive colonies are inoculated in 3 ml LB 100 μ g/ml Ampicillin and grown at 37 °C overnight. 70 μ l of the overnight culture is inoculated in 2 ml LB/Amp and grown at 37 °C until OD₆₀₀ of the pET clones reached the 0,4-0,8 value or until OD₆₀₀ of the pGEX clones reached the 0,8-1 value. Protein expression is then induced by adding 1 mM IPTG (Isopropil β -D thio-galacto-piranoside) to the mini-cultures. After 3 hours incubation at 37 °C the final OD₆₀₀ is checked and the cultures are cooled on ice. After centrifugation of 0.5 ml culture, the cell pellet is suspended in 50 μ l of protein Loading Sample Buffer (60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% w/v Bromophenol Blue, 100 mM DTT) and incubated at 100 °C for 5 min. A volume of boiled sample corresponding to 0.1 OD₆₀₀ culture is analysed by SDS-PAGE and Coomassie Blue staining to verify the presence of induced protein band.

3. Purification of the recombinant proteins

Single colonies are inoculated in 25 ml LB 100 μ g/ml Ampicillin and grown at 37 °C overnight. The overnight culture is inoculated in 500 ml LB/Amp and grown under shaking at 25 °C until OD₆₀₀ 0.4-0.7. Protein expression is then induced by adding 1 mM IPTG to the cultures. After 3.5 hours incubation at 25 °C the final OD₆₀₀ is checked and the cultures are cooled on ice. After centrifugation at 6000 rpm (JA10 rotor, Beckman), the cell pellet is processed for purification or frozen at -20° C.

(a) *Procedure for the purification of soluble His-tagged proteins from E.coli*

- (1) Transfer the pellets from -20°C to ice bath and reconstitute with 10 ml 50 mM NaHPO_4 buffer, 300 mM NaCl, pH 8.0, pass in 40-50 ml centrifugation tubes and break the cells as per the following outline.
- 5 (2) Break the pellets in the French Press performing three passages with in-line washing.
- (3) Centrifuge at about 30-40000 x g per 15-20 min. If possible use rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.)
- (4) Equilibrate the Poly-Prep columns with 1 ml Fast Flow Chelating Sepharose resin with 50 mM phosphate buffer, 300 mM NaCl, pH 8.0.
- 10 (5) Store the centrifugation pellet at -20°C , and load the supernatant in the columns.
- (6) Collect the flow through.
- (7) Wash the columns with 10 ml (2 ml + 2 ml + 4 ml) 50 mM phosphate buffer, 300 mM NaCl, pH 8.0.
- (8) Wash again with 10 ml 20 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8.0.
- 15 (9) Elute the proteins bound to the columns with 4.5 ml (1.5 ml + 1.5 ml + 1.5 ml) 250 mM imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8.0 and collect the 3 corresponding fractions of ~1.5 ml each. Add to each tube 15 μl DTT 200 mM (final concentration 2 mM)
- (10) Measure the protein concentration of the first two fractions with the Bradford method, collect a 10 μg aliquot of proteins from each sample and analyse by SDS-PAGE. (N.B.: should the sample be
- 20 too diluted, load 21 μl + 7 μl loading buffer).
- (11) Store the collected fractions at $+4^{\circ}\text{C}$ while waiting for the results of the SDS-PAGE analysis.
- (12) For immunisation prepare 4-5 aliquots of 100 μg each in 0.5 ml in 40% glycerol. The dilution buffer is the above elution buffer, plus 2 mM DTT. Store the aliquots at -20°C until immunisation.

(b) *Purification of His-tagged proteins from Inclusion bodies*

- 25 Purifications are carried out essentially according the following protocol:
- (1) Bacteria are collected from 500 ml cultures by centrifugation. If required store bacterial pellets at -20°C . For extraction, resuspend each bacterial pellet in 10 ml 50 mM TRIS-HCl buffer, pH 8.5 on an ice bath.
- (2) Disrupt the resuspended bacteria with a French Press, performing two passages.
- 30 (3) Centrifuge at 35000 x g for 15 min and collect the pellets. Use a Beckman rotor JA 25.50 (21000 rpm, 15 min.) or JA-20 (18000 rpm, 15 min.).
- (4) Dissolve the centrifugation pellets with 50 mM TRIS-HCl, 1 mM TCEP {Tris(2-carboxyethyl)-phosphine hydrochloride, Pierce}, 6M guanidium chloride, pH 8.5. Stir for ~ 10 min. with a magnetic bar.
- 35 (5) Centrifuge as described above, and collect the supernatant.
- (6) Prepare an adequate number of Poly-Prep (Bio-Rad) columns containing 1 ml of Fast Flow Chelating Sepharose (Pharmacia) saturated with Nickel according to manufacturer recommendations..

Wash the columns twice with 5 ml of H₂O and equilibrate with 50 mM TRIS-HCl, 1 mM TCEP, 6M guanidinium chloride, pH 8.5.

(7) Load the supernatants from step 5 onto the columns, and wash with 5 ml of 50 mM TRIS-HCl buffer, 1 mM TCEP, 6M urea, pH 8.5

5 (8) Wash the columns with 10 ml of 20 mM imidazole, 50 mM TRIS-HCl, 6M urea, 1 mM TCEP, pH 8.5. Collect and set aside the first 5 ml for possible further controls.

(9) Elute the proteins bound to the columns with 4.5 ml of a buffer containing 250 mM imidazole, 50 mM TRIS-HCl, 6M urea, 1 mM TCEP, pH 8.5. Add the elution buffer in three 1.5 ml aliquots, and collect the corresponding 3 fractions. Add to each fraction 15 µl DTT (final concentration 2 mM).

10 (10) Measure eluted protein concentration with the Bradford method, and analyse aliquots of ca 10 µg of protein by SDS-PAGE.

(11) Store proteins at -20°C in 40% (v/v) glycerol, 50 mM TRIS-HCl, 2M urea, 0.5 M arginine, 2 mM DTT, 0.3 mM TCEP, 83.3 mM imidazole, pH 8.5.

(c) *Procedure for the purification of GST-fusion proteins from E.coli*

15 (1) Transfer the bacterial pellets from -20°C to an ice bath and suspend with 7,5 ml PBS, pH 7,4 to which a mixture of protease inhibitors (COMPLETE™ - Boehringer Mannheim, 1 tablet every 25 ml of buffer) has been added.

(2) Transfer to 40-50 ml centrifugation tubes and sonicate according to the following procedure:

- 20 a. Position the probe at about 0,5 cm from the bottom of the tube
b. Block the tube with the clamp
c. Dip the tube in an ice bath
d. Set the sonicator as follows: Timer → Hold, Duty Cycle → 55, Out. Control → 6.
e. perform 5 cycles of 10 impulses at a time lapse of 1 minute (i.e. one cycle = 10 impulses + ~45" hold; b. 10 impulses + ~45" hold; c. 10 impulses + ~45" hold; d. 10 impulses + ~45" hold; e. 10
25 impulses + ~45" hold).

(3) Centrifuge at about 30-40000 x g for 15-20 min. E.g.: use rotor Beckman JA 25.50 at 21000 rpm, for 15 min.

30 (4) Store the centrifugation pellets at -20°C, and load the supernatants on the chromatography columns, as follows

(5) Equilibrate the Poly-Prep (Bio-Rad) columns with 0,5 ml (≅ 1 ml suspension) of Glutathione-Sepharose 4B resin, wash with 2 ml (1 + 1) H₂O, and then with 10 ml (2 + 4 + 4) PBS, pH 7,4.

(6) Load the supernatants on the columns and discard the flow through.

(7) Wash the columns with 10 ml (2 + 4 + 4) PBS, pH 7.4.

35 (8) Elute the proteins bound to the columns with 4.5 ml of 50 mM TRIS buffer, 10 mM reduced glutathione, pH 8.0, adding 1.5 ml + 1.5 ml + 1.5 ml and collecting the respective 3 fractions of ~1.5 ml each.

(9) Measure the protein concentration of the first two fractions with the Bradford method, analyse a 10 µg aliquot of proteins from each sample by SDS-PAGE. (N.B.: if the sample is too diluted load 21 µl (+ 7 µl loading buffer).

(10) Store the collected fractions at +4°C while waiting for the results of the SDS-PAGE analysis.

5 (11) For each protein destined to the immunisation prepare 4-5 aliquots of 100 µg each in 0.5 ml of 40% glycerol. The dilution buffer is 50 mM TRIS.HCl, 2 mM DTT, pH 8.0. Store the aliquots at -20°C until immunisation.

4. Murine Model of Protection from GAS Infection

(a) *Immunization protocol*

10 Groups of 10 CD1 female mice aged between 6 and 7 weeks are immunized with two or more GAS antigens of the invention, (20 µg of each recombinant GAS antigen), suspended in 100 µl of suitable solution. Each group receives 3 doses at days 0, 21 and 45. Immunization is performed through intra-peritoneal injection of the protein with an equal volume of Complete Freund's Adjuvant (CFA) for the first dose and Incomplete Freund's Adjuvant (IFA) for the following two doses. In each immunization
15 scheme negative and positive control groups are used.

For the negative control group, mice are immunized with *E. coli* proteins eluted from the purification columns following processing of total bacterial extract from a *E. coli* strain containing either the pET21b or the pGEX-NNH vector (thus expressing GST only) without any cloned GAS ORF (groups can be indicated as HisStop or GSTStop respectively).

20 For the positive control groups, mice are immunized with purified GAS M cloned from either GAS SF370 or GAS DSM 2071 strains (groups indicated as 192SF and 192DSM respectively).

Pooled sera from each group is collected before the first immunization and two weeks after the last one. Mice are infected with GAS about a week after.

Immunized mice are infected using a GAS strain different from that used for the cloning of the
25 selected proteins. For example, the GAS strain can be DSM 2071 M23 type, obtainable from the German Collection of Microorganisms and Cell Cultures (DSMZ).

For infection experiments, DSM 2071 is grown at 37° C in THY broth until OD₆₀₀ 0.4. Bacteria are pelleted by centrifugation, washed once with PBS, suspended and diluted with PBS to obtain the appropriate concentration of bacteria/ml and administered to mice by intraperitoneal injection.

30 Between 50 and 100 bacteria are given to each mouse, as determined by plating aliquots of the bacterial suspension on 5 THY plates. Animals are observed daily and checked for survival.

5. Analysis of Immune Sera

(a) *Preparation of GAS total protein extracts*

Total protein extracts are prepared by incubating a bacterial culture grown to OD₆₀₀ 0.4-0.5 in Tris
35 50mM pH 6.8/mutanolysin (20 units/ml) for 2 hr at 37° C, followed by incubation for ten minutes on ice in 0.24 N NaOH and 0.96% β-mercaptoethanol. The extracted proteins are precipitated by addition of trichloroaceticacid, washed with ice-cold acetone and suspended in protein loading buffer.

(b) *Western blot analysis*

Aliquots of total protein extract mixed with SDS loading buffer (1x: 60 mM TRIS-HCl pH 6.8, 5% w/v SDS, 10% v/v glycerin, 0.1% Bromophenol Blue, 100 mM DTT) and boiled 5 minutes at 95° C, were loaded on a 12.5% SDS-PAGE precast gel (Biorad). The gel is run using a SDS-PAGE running
5 buffer containing 250 mM TRIS, 2.5 mM Glycine and 0.1 %SDS. The gel is electroblotted onto nitrocellulose membrane at 200 mA for 60 minutes. The membrane is blocked for 60 minutes with PBS/0.05 % Tween-20 (Sigma), 10% skimmed milk powder and incubated O/N at 4° C with PBS/0.05 % Tween 20, 1% skimmed milk powder, with the appropriate dilution of the sera. After washing twice with PBS/0.05 % Tween, the membrane is incubated for 2 hours with peroxidase-
10 conjugated secondary anti-mouse antibody (Amersham) diluted 1:4000. The nitrocellulose is washed three times for 10 minutes with PBS/0.05 % Tween and once with PBS and thereafter developed by Opti-4CN Substrate Kit (Biorad).

(c) *Preparation of Paraformaldehyde treated GAS cultures*

A bacterial culture grown to OD₆₀₀ 0.4-0.5 is washed once with PBS and concentrated four times in
15 PBS/0.05 % Paraformaldehyde. Following 1 hr incubation at 37° C with shaking, the treated culture is kept overnight at 4° C and complete inactivation of bacteria is then controlled by plating aliquots on THY blood agar plates.

(d) *FACS analysis of Paraformaldehyde treated GAS cultures with mouse immune sera*

About 10⁵ Paraformaldehyde inactivated bacteria are washed with 200 µl of PBS in a 96 wells U
20 bottom plate and centrifuged for 10 min. at 3000g, at 4°C. The supernatant is discarded and the bacteria are suspended in 20 µl of PBS-0.1%BSA. Eighty µl of either pre-immune or immune mouse sera diluted in PBS-0.1%BSA are added to the bacterial suspension to a final dilution of either 1:100, 1:250 or 1:500, and incubated on ice for 30 min. Bacteria are washed once by adding 100 µl of PBS-0.1%BSA, centrifuged for 10 min. at 3000g, 4°C, suspended in 200 µl of PBS-0.1%BSA, centrifuged
25 again and suspended in 10 µl of Goat Anti-Mouse IgG, F(ab')₂ fragment specific-R-Phycoerythrin-conjugated (Jackson ImmunoResearch Laboratories Inc., cat.N°115-116-072) in PBS-0.1%BSA to a final dilution of 1:100, and incubated on ice for 30 min. in the dark. Bacteria are washed once by adding 180 µl of PBS-0.1%BSA and centrifuged for 10 min. at 3000g, 4°C. The supernatant is discarded and the bacteria were suspended in 200 µl of PBS. Bacterial suspension is passed through a
30 cytometric chamber of a FACS Calibur (Becton Dickinson, Mountain View, CA USA) and 10.000 events are acquired. Data are analysed using Cell Quest Software (Becton Dickinson, Mountain View, CA USA) by drawing a morphological dot plot (using forward and side scatter parameters) on bacterial signals. An histogram plot is then created on FL2 intensity of fluorescence log scale recalling the morphological region of bacteria.

35 It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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coiled coil

leucine zipper

Transmembrane region

FIGURE 2 : Schematic of GAS40: putative surface exclusion protein prgA (873aa)

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